Applicants: Zhongyi Li et al.
Serial No.: 10/577,564
Filed: April 26, 2006
Exhibit 1

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/54, 15/11, 9/10, C12Q 1/48, 1/68, A01H 1/00, 5/00, C08B 3/02

A1

(11) International Publication Number: WO 00/66745

(43) International Publication Date: 9 November 2000 (09.11.00)

(21) International Application Number: PCT/AU00/00385

(22) International Filing Date: 28 April 2000 (28.04.00)

(30) Priority Data:
PQ0052/99 29 April 1999 (29.04.99) AU

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(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: NOVEL GENES ENCODING WHEAT STARCH SYNTHASES AND USES THEREFOR

(57) Abstract

The present invention provides isolated nucleic acid molecules encoding wheat starch synthases, and probes and primers derived therefrom, which are useful in the modificaiton of plant starch content and/or composition, and for screening plant lines to determine the presence of natural and/or induced mutations in starch synthase genes which affect starch content and/or composition. More particularly, the isolated nucleic acid molecules of the present invention further provide for the screening-assisted breeding of plants having desirable starch content and/or composition, in addition to providing for the direct genetic manipulation of plant starch content and/or composition.

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NOVEL GENES ENCODING WHEAT STARCH SYNTHASES AND USES THEREFOR

FIELD OF THE INVENTION

The present invention relates generally to isolated nucleic acid molecules encoding wheat starch synthase enzymes and more particularly, to isolated nucleic acid molecules that encode wheat SSII and SSIII enzyme activities. The isolated nucleic acid molecules provide the means for modifying starch content and composition in plants, for example the ratio of amylose:amylopectin in the starch granule of the endosperm during the grain-filling phase of endosperm development. The isolated nucleic acid molecules of the present invention also provide the means for screening plant lines to determine the presence of natural and/or induced mutations in starch synthase genes which affect starch content and/or composition. The isolated nucleic acid molecules of the present invention further provide for the screening-assisted breeding of plants having desirable starch content and/or composition, in addition to providing for the direct genetic manipulation of plant starch content and/or composition.

GENERAL

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Reference herein to any published document is not to be taken as an indication or admission that any such published document is part of the common general knowledge or background information of a skilled worker in the relevant field.

This specification contains nucleotide and amino acid sequence information (SEQ ID NOS:) prepared using the programme PatentIn Version 2.0, presented herein at the end of the specification. Each nucleotide or amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, etc). The length, type of sequence (DNA, protein (PRT), etc) and source organism for each nucleotide or amino acid sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>,

respectively. Nucleotide and amino acid sequences (SEQ ID NOs:) referred to in the specification are defined by the information provided in numeric indicator field <400> followed by the sequence identifier (eg. SEQ ID NO: 1 is <400>1, etc).

The designation of nucleotide residues referred to herein are those recommended by the IUPAC-IUB Biochemical Nomenclature Commission, wherein A represents Adenine, C represents Cytosine, G represents Guanine, T represents thymine, Y represents a pyrimidine residue, R represents a purine residue, M represents Adenine or Cytosine, K represents Guanine or Thymine, S represents Guanine or Cytosine, W represents Adenine or Thymine, H represents a nucleotide other than Guanine, B represents a nucleotide other than Adenine, V represents a nucleotide other than Thymine, D represents a nucleotide other than Cytosine and N represents any nucleotide residue.

The designations for naturally-occurring amino acid residues referred to herein are set forth in Table I. The designations for a non-limiting set of non-naturally-occurring amino acids is listed in Table 2.

As used herein the term "derived from" shall be taken to indicate that a specified integer may be obtained from a particular source albeit not necessarily directly from that source.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of steps or elements or integers.

TABLE 1

Amino Acid	Three-letter Code	One-letter Code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Aspartate/glutamate	Baa	B
Asparagine/glutamine		
Any amino acid as above	Xaa	X

TABLE 2

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva

D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl) glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl) glycine	Nbhe

D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl) glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)) glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl) glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomo phenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl) glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys

L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	Mser	L-α-methylthreonine	Mthr
L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
L-α-methylvaline	Mval	L-N-methylhomo phenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purposes of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

BACKGROUND TO THE INVENTION

The biosynthesis of the starch granule is a complex process which involves the action of an array of isoforms of enzymes involved in the starch biosynthesis. Following the formation of glucose-1-phosphate, the enzyme activities required for the synthesis of granular starch include ADP glucose pyrophosphorylase (EC 2.7.7.27), starch synthases (EC 2.4.1.21), branching enzymes (EC 2.4.1.18) and debranching enzymes

(EC 3.2.1.41 and EC 3.2.1.68) (Mouille *et al.*, 1996). Plants contain isozymes of each of these activities, and the definition of these isoforms and their roles has been conducted through investigation of the properties of the suite of soluble enzymes found in the stroma of the plastid, analysis of the proteins entrapped within the matrix of the starch granule, and mutational studies to identify genes and define linkages between individual genes and their specific roles.

Starch synthases extend regions of α-1,4 glucan through the transfer of the glucosyl moiety of ADPglucose to the non-reducing end of a pre-existing α-1,4 glucan. In addition to GBSS, 3 other classes of starch synthase have been identified in plants, SSI (wheat, Li *et al.*, 1999 and GenBank Accession No. U48227; rice, Baba *et al.*, 1993; potato, Genbank Accession No. STSTASYNT), SSII (pea, Dry *et al.* 1992; potato, Edwards *et al.*, 1995; maize, Harn *et al.* 1998 and GenBank Accession No. U66377) and SSIII (potato, Abel *et al.*, 1996; maize, Gao *et al.*, 1998). In the cereals, the most comprehensively studied species is maize, where in addition to GBSS, cDNAs encoding SSI, SSIIa, and SSIIb have been isolated, and both cDNA and genomic clones for *dull*1 have been characterised (Knight *et al.*, 1998; Harn *et al.*, 1998; Gao *et al.*, 1998). In maize, the product of the *du1* gene is known as maize SSII, however this gene is the homologue of potato SSIII.

The proteins within the matrix of the wheat starch granule have been extensively studied (Denyer *et al.*, 1995; Rahman *et al.*, 1995; Takaoka *et al.*, 1997; Yamamori and Endo, 1996) and 60, 75, 85, 100, 104 and 105 kDa protein bands can be visualised following SDS-PAGE. The predominant 60 kDa protein is exclusively granule-bound and is analogous to the "waxy" granule bound starch synthase (GBSS) gene in maize (Rahman *et al.*, 1995). The combination of three null alleles for this enzyme from each of the wheat genomes (Nakamura *et al.*, 1995) results in the amylose-free "waxy" phenotype found in other species The 75 kDa starch synthase I (wSSI) is found in both the granule and the soluble fraction of wheat endosperm (Denyer *et al.*, 1995; Li *et al.*, 1999) and has been assigned to chromosomes 7A, 7B and 7D (Yamamori and Endo, 1996; Li *et al.*, 1999). The 85 kDa band contains a

class II branching enzyme and an unidentified polypeptide (Rahman *et al.*, 1995). The 100, 104 and 105 kDa proteins of the wheat starch granule (designated Sgp-B1, Sgp-D1 and Sgp-A1 by Yamamori and Endo, 1996) have been shown to be encoded by a homeologous set of genes on the short arm of chromosome 7B, 7A and 7D respectively (Yamamori and Endo, 1996; Takaoka *et al.*, 1997). Denyer *et al.* (1995) concluded on the basis of enzyme activity assays that these proteins were also starch synthases. These genes are referred to hereinafter as the "wheat SSII genes".

While GBSS has been established to be essential for amylose synthesis, the remaining starch synthases are thought to be primarily responsible for the elongation of amylopectin chains, although this does not preclude them from also having non-essential roles in amylose biosynthesis. Differences in kinetic properties between isoforms, and the analysis of mutants lacking various isoforms, suggests that each isoenzyme contributes to the extension of specific subsets of the available non-reducing ends.

SUMMARY OF THE INVENTION

The production of plants that produce improved starches that are modified for particular end-use applications, such as, for example, starches having high or low amylose:amylopectin ratios, requires the availability of genes encoding the various starch synthase isoforms. Because of species-specific codon usages, and variations in the kinetic parameters of the starch synthase isoforms between species, the production of modified starches may require the use of genes derived from particular species.

Furthermore, the screening-assisted breeding of plants having desirable starch content and/or composition requires specific gene sequences to be provided that can be used to distinguish between different homeologous genes encoding the various isoforms of wheat starch synthases, such as, for example, to identify and distinguish between naturally-occurring variant gene sequences. It is a particular object of the present invention to provide gene sequences to facilitate the screening-assisted selection of

wheat plants having starch traits which are associated with the presence and/or expression of one or more wheat SSI and/or SSIII genes.

Accordingly, the present invention provides isolated nucleotide sequences encoding the wheat SSII (i.e. wSSII) and wheat SSIII (i.e. wSSIII) isoenzymes, and DNA markers derived therefrom. The present invention further facilitates the production of transformed plants carrying these nucleotide sequences.

More particularly, the present invention provides isolated nucleic acid molecules encoding the 100, 104 and 105 kDa SSII (Sgp-1) polypeptides of the wheat starch granule matrix, as determined using the SDS/PAGE system of Rahman *et al.* (1995), which polypeptides are equivalent to the 100, 108 and 115 kDa polypeptides described by Yamamori and Endo (1996).

The present invention further provides isolated nucleic acid molecules encoding the soluble *dull*1-type wheat starch synthase III polypeptide. Analysis of the polypeptides encoded by these nucleic acid molecules reveals several consensus amino acid sequence motifs that are highly conserved in wheat starch synthase isoenzymes, in addition to isoenzyme-specific sequences, which sequences possess utility in isolating related starch synthase-encoding sequences and in assaying plants for their expression of one or more starch synthase isoenzymes.

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides which encodes, or is complementary to a nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof selected from the following:

(i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;

(ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;

(iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

(a) KVGGLGDVVTS (SEQ ID NO: 39);

(b) GHTVEVILPKY (SEQ ID NO: 40);

(c) HDWSSAPVAWLYKEHY (SEQ ID NO: 41);

(d) GILNGIDPDIWDPYTD (SEQ ID NO: 42);

(e) DVPIVGIITRLTAQKG (SEQ ID NO: 43);

(f) NGQVVLLGSA (SEQ ID NO: 44);

(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS (SEQ ID NO: 45); and

(h)TGGLVDTV (SEQ ID NO: 46);

wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10; and

(iv) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

(a) KTGGLGDVAGA (SEQ ID NO: 47);

(b) GHRVMVVVPRY (SEQ ID NO: 48);

(c) NDWHTALLPVYLKAYY (SEQ ID NO: 49);

(d) GIVNGIDNMEWNPEVD (SEQ ID NO: 50);

(e) DVPLLGFIGRLDGQKG (SEQ ID NO: 51);

(f) DVQLVMLGTG (SEQ ID NO: 52);

(g)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT (SEQ ID NO: 53); and

(h)VGG(V/L)RDTV (SEQ ID NO: 54);

wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10.

In a preferred embodiment, the isolated nucleic acid molecule encodes a starch synthase polypeptide, protein or enzyme having at least about 90% amino acid sequence identity to any one of SEQ ID NOS: 2, 4, 6, 8 or 10, more preferably having at least about 95% or about 97% or about 99% identity to any one of said amino acid sequences.

In an alternative embodiment, the isolated nucleic acid molecule of the present invention encodes a wheat starch synthase polypeptide which comprises one or more amino acid sequences selected from the group consisting of:

(a) GHTVEVILPKY;
(b) HDWSSAPVAWLYKEHY;
(c) DVPIVGIITRLTAQKG;
(d) NGQVVLLGSA;
(e)AGSDFIIVPSIFEPCGLTQLVAMRYGS;
(f)TGGLVDTV;
(g) GIVNGIDNMEWNPEVD; and
(h) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT.

In an alternative embodiment, the present invention provides an isolated nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof, wherein said nucleic acid molecule comprises a nucleotide sequence having at least about 85% nucleotide sequence identity to any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37 or 38 or a complementary nucleotide sequence thereto.

In a preferred embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37 or 38,

or is at least about 90% identical, more preferably at least about 95% or 97% or 99% identical to all or a protein-encoding part thereof.

In an alternative embodiment, the present invention provides an isolated nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof, wherein said nucleic acid molecule comprises a nucleotide sequence that is capable of hybridising under at least moderate stringency hybridisation conditions to at least about 30 contiguous nucleotides derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37 or 38, or a complementary nucleotide sequence thereto.

A second aspect of the present invention provides a method of isolating a nucleic acid molecule that encodes a starch synthase polypeptide, protein or enzyme described *supra,* said method comprising:

(i) hybridising a probe or primer comprising at least about 15 contiguous nucleotides in length derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37 or 38, or a complementary nucleotide sequence thereto to single-stranded or double-stranded mRNA, cDNA or genomic DNA; and

(ii) detecting the hybridised mRNA, cDNA or genomic DNA using a detecting means.

Preferably, the detecting means is a reporter molecule covalently attached to the probe or primer molecule or alternatively, a polymerase chain reaction format. Accordingly, the present invention clearly extends to the use of the nucleic acid molecules provided herein to isolate related starch synthase-encoding sequences using standard hybridisation and/or polymerase chain reaction techniques.

A third aspect of the invention provides an isolated probe or primer comprising at least about 15 contiguous nucleotides in length derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37 or 38, or a complementary nucleotide sequence thereto.

Preferably, the probe or primer comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 25 to 34.

A fourth aspect of the present invention is directed to an isolated or recombinant starch synthase polypeptide, protein or enzyme, preferably substantially free of conspecific or non-specific proteins, which comprises an amino acid sequence selected from the following:

(i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;

(ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;

(iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

(a) KVGGLGDVVTS;

(b) GHTVEVILPKY;

(c) HDWSSAPVAWLYKEHY;

(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVVLLGSA;

(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS; and

(h)TGGLVDTV

wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10; and

(iv) a wheat starch synthase polypeptide, protein or enzyme or functional

subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

(a) KTGGLGDVAGA;

(b) GHRVMVVVPRY;

(c) NDWHTALLPVYLKAYY;

(d) GIVNGIDNMEWNPEVD;

(e) DVPLLGFIGRLDGQKG;

(f) DVQLVMLGTG;

(g)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(h)VGG(V/L)RDTV

wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10.

The present invention clearly encompasses the mature protein region of a wheat starch synthase polypeptide which is obtained by removal of the N-terminal transit peptide sequence.

A further aspect of the invention provides a method of assaying for the presence or absence of a starch synthase isoenzyme or the copy number of a gene encoding same in a plant, comprising contacting a biological sample derived from said plant with an isolated nucleic acid molecule derived from any one of SEQ ID NOS 1, 3, 5, 7, 9,11-16, 37 or 38, or any one of SEQ ID NOS: 25 to 34, or a complementary nucleotide sequence thereto for a time and under conditions sufficient for hybridisation to occur and then detecting said hybridisation using a detection means.

The detection means according to this aspect of the invention is any nucleic acid based hybridisation or amplification reaction.

A further aspect of the present invention utilises the above-mentioned assay method

in the breeding and/or selection of plants which express or do not express particular starch synthase isoenzymes or alternatively, which express a particular starch synthase isoenzyme at a particular level in one or more plant tissues. This aspect clearly extends to the selection of transformed plant material which contains one or more of the isolated nucleic acid molecules of the present invention.

A further aspect of the present invention provides a method of modifying the starch content and/or starch composition of one or more tissues or organs of a plant, comprising expressing therein a sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37 or 38, or a complementary nucleotide sequence thereto for a time and under conditions sufficient for the enzyme activity of one or more starch synthase isoenzymes to be modified. This aspect of the invention clearly extends to the introduction of the sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule to isolated plant cells, tissues or organs or organelles by cell fusion or transgenic means and the regeneration of intact plants therefrom.

A further aspect of the present invention provides an isolated promoter that is operable in the endosperm of a monocotyledonous plant cell, tissue or organ, and preferably in the endosperm of a monocotyledonous plant cell, tissue or organ. For example, the HMG promoter from wheat, or the maize zein gene promoter are particularly preferred, as is the promoter derived from a starch synthase gene of the present invention, such as a promoter that is linked *in vivo* to any one of SEQ ID NOS 1, 3, 5, 7, 9,11-16, 37 or 38, or a complementary nucleotide sequence thereto.

A still further aspect of the present invention contemplates a transgenic plant comprising an introduced sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16,

37 or 38, or a complementary nucleotide sequence thereto or a genetic construct comprising same, and to plant propagules, cells, tissues, organs or plant parts derived from said transgenic plant that also carry the introduced molecule(s).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a copy of a photographic representation showing the distribution of wheat endosperm starch synthases between the starch granule and soluble fractions. Lane 1, SDS-PAGE of wheat endosperm starch granule proteins revealed by silver staining; lanes 2-7, immunoblot of wheat endosperm soluble phase and starch granule proteins separated by SDS-PAGE from various developmental stages and probed with an anti-(wheat wSSII peptide) monoclonal antibody. Lanes 2-4 contain proteins from the soluble fraction of wheat endosperm at 15 days post anthesis (Lane 2); 20 days post anthesis (Lane 3); and at 25 days post anthesis (Lane 4). Lanes 5-7 contain proteins from the starch granule of wheat endosperm at 15 days post anthesis (Lane 5); 20 days post anthesis (Lane 6); and at 25 days post anthesis (Lane 7).

Figure 2 is a copy of a schematic representation comparing the nucleotide sequences of cDNA clones designated wSSIIA, wSSIIB and wSSIID, encoding the starch synthase II polypeptides from wheat, using the PILEUP programme of Devereaux *et al.* (1984).

Figure 3 is a copy of a schematic representation comparing the deduced amino acid sequences of starch synthase II from wheat (wSSIIA, wSSIIB and wSSIID), maize (maize SSIIa and maize SSIIb; Harn *et al.*, 1998), pea (pea SSII; Dry et al ., 1992) and potato (potato SSII; van der Leij *et al.*, 1991). Identical amino acid residues among each of these sequences are indicated below the sequences with "*". The alignments of maize SSIIa with maize SSIIb, and pea SSII and potato SSII are essentially as described in Harn *et al.* (1998) and Edwards *et al.* (1995). All sequences are aligned to position the transit peptide cleavage site below the arrow (⇩) between residues 59 and 60 of the wSSIIA sequence. The wSSIIp1 sequence, the sequence of SGP-B1 (peptide3), and of eight conserved regions are annotated and underlined.

Figure 4 is a copy of a photographic representation of a northern blot showing the expression of wheat wSSII mRNA in wheat plants. Total RNAs were isolated from leaves pre-anthesis florets and endosperm of the wheat cultivar "Gabo", grown under a photoperiod comprising 16 hours daylength, and at 18 °C during the day, and at 13 °C during the night cycle, and probed with the wSSIIp2 DNA fragment. The source of each RNA is indicated at the top of the Figure as follows: Lane 1, leaf; Lane 2, pre-anthesis florets; Lanes 3-11, endosperm at: 4 days post-anthesis (Lane 3); 6 days post-anthesis (Lane 4); 8 days post-anthesis (Lane 5); 10 days post-anthesis (Lane 6);12 days post-anthesis (Lane 7); 15 days post-anthesis (Lane 8); 18 days post-anthesis (Lane 9); 21 days post-anthesis (Lane 10); and 25 days post-anthesis (Lane 11).

Figure 5 is a copy of a photographic representation showing the localization of wheat starch synthase II genes on the wheat genome by PCR, using the primers sslIc, sslId and sslIe in the amplification reaction. The nullisomic-tetrasomic genomic DNA of wheat cv. Chinese Spring was used as template DNA. Lane D, *Triticum tauschii*; Lane AB, Accession line N7DT7B having no 7D chromosome and four copies of the 7B chromosome; Lane AD, Accession line N7BT7A having no 7B chromosome and four copies of the 7A chromosome; Lane BD, Accession line N7AT7B having no 7A chromosome and four copies of the 7B chromosome; Lane ABD, wheat cv. Chinese Spring. PCR products derived from each cDNA clone are labelled. The results indicate that the cDNA clones, wSSIIB, wSSIIA and wSSIID are derived from the B-, A- and D-genomes of wheat, respectively.

Figure 6 is a schematic representation showing the organisation of introns (lines) and exons (boxes) in the wheat SSII gene shown in SEQ ID NO: 37. The scale (bases), relative to the nucleotide sequence set forth in SEQ ID NO: 37, is provided at the bottom of the figure.

Figure 7 is a schematic representation comparing the deduced amino acid Sequences of the maize, potato and wheat SSIII polypeptides.

Figure 8 is a copy of a photographic representation showing the expression of wheat wSSIII mRNA in wheat. Total RNAs were isolated from the endosperm of the wheat cultivars Wyuna (Panel a) and Gabo (Panel b) leaves pre-anthesis florets and endosperm of the wheat cultivar "Gabo", grown under a photoperiod comprising 16 hours daylength, and at 18 °C during the day cycle, and at 13 °C during the night cycle, and probed with the wSSIIIp1 DNA fragment derived from wSSIII.B3 cDNA. The source of each RNA is indicated at the top of the Figure as follows: Lane 1, endosperm at: 4 days post-anthesis; Lane 2, endosperm at 6 days post-anthesis; Lane 4, endosperm at 8 days post-anthesis; Lane 4, endosperm at 10 days post-anthesis; Lane 5, endosperm at 12 days post-anthesis; Lane 6, endosperm at 15 days post-anthesis; Lane 7, endosperm at 18 days post-anthesis; Lane 8, endosperm at 21 days post-anthesis; Lane 9, endosperm at 25 days post-anthesis; and Lane 10, endosperm at 31 days post-anthesis (Panel a only). In panel (c), L refers to leaf RNA, and P refers to RNA from pre-anthesis florets derived from the cultivar Gabo.

Figure 9 is a schematic representation showing the position of conserved amino acid sequences within four wheat starch synthase proteins. The eight highly-conserved regions between the wheat starch synthase polypeptides are underlined and annotated at the top of each group of amino acid sequences. The sequences included in the alignment are the wheat SSII-A1 and wheat SSIII polypeptides of the present invention; wheat GBSS (wGBSS; Yan *et al.*, 1999); wheat SSI (wSS1; Li *et al.*, 1999); wheat SSII (wSS2; SEQ ID NO: 4); and wheat SSIII (wSS3; SEQ ID NO: 8).

Figure 10 is a schematic representation showing the relationships between the primary amino acid sequences of starch synthases (SS) and glycogen synthase of *E. coli* (GS). The dendrogram was generated by the program PILEUP (Devereaux *et al.*, 1984). The amino acid sequences used for the analysis are those of the wheat SSIIA, wheat SSIIB, wheat SSIID, and wheat SSIII polypeptides of the present invention compared to the deduced amino acid sequences of wheat GBSS (Clark *et al.*, 1991), wheat SSI (Li *et al.*, 1999), rice GBSS (Okagaki, 1992), rice SSI (Baba *et al.*, 1993), maize GBSS (Kloesgen *et al.*, 1986), maize SSI (Knight *et al.*, 1998), maize SSIIa and

maize SSIIb (Harn *et al.*, 1998), maize SSIII (Gao *et al.*, 1998), pea GBSS (Dry *et al.*, 1992), pea SSII (Dry *et al.*, 1992), potato GBSS (van der Leij *et al.*, 1991), potato SSI (Genbank accession number: STSTASYNT), potato SSII (Edwards *et al.*, 1995), potato SSIII (Abel *et al.*, 1996), and *E. coli* glycogen synthase (GS) (Kumar *et al.*, 1986). Five groups of enzymes included in the alignment are granule-bound starch synthase (GBSS), starch synthase-I (SSI), starch synthase-II (SSII), starch synthase-III (SSIII) and glycogen synthase (GS).

Figure 11 is a schematic representation showing the position of conserved regions within cereal starch synthase genes. Comparisons of cereal starch synthases were made based on their deduced amino acid sequences and 8 conserved regions identified. Conserved regions are shown in bold and transit peptides (where defined) in grey. The sequences included in the alignment are the wheat SSII-A1 and wheat SSIII polypeptides of the present invention; wheat GBSS (Ainsworth *et al.*, 1993); wheat SSI (Li *et al.*, 1999); maize SSIIa (Harn *et al.*, 1998); and maize dull-1(Gao *et al.*, 1998).

Figure 12 is a copy of a schematic representation of a gene map showing the alignment of fragments 1 to 6 of the genomic SSIII gene (lower line) with the corresponding SSIII cDNA clone (upper line). Raised regions in the genomic clone fragments (lower line) represent protein-encoding regions of the gene.

Figure 13 is a schematic representation showing the organisation of introns (lines) and exons (boxes) in the wheat SSIII gene shown in SEQ ID NO: 38. The scale (bases), relative to the nucleotide sequence set forth in SEQ ID NO: 38, is provided at the bottom of the figure.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

One aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides which encodes, or is complementary to a nucleic

acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof selected from the following:

(i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6; and

(ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10.

Alternatively or in addition, the isolated nucleic acid molecule of the present invention encodes a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof and comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, 5, or 37.

Alternatively or in addition, the isolated nucleic acid molecule of the present invention encodes a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof and comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 7, 9, or 38.

As used herein, the term "starch synthase" shall be taken to refer to any enzymatically-active peptide, polypeptide, oligopeptide, polypeptide, protein or enzyme molecule that is at least capable of transferring a glucosyl moiety from ADP-glucose to an α-1,4-glucan molecule, or a peptide, polypeptide, oligopeptide or polypeptide fragment of such an enzymatically-active molecule.

The term "wheat starch synthase" refers to a starch synthase derived from hexaploid wheat or barley or a progenitor species, or a relative thereto such as the diploid *Triticum tauschii* or other diploid, tetraploid, aneuploid, polyploid, nullisomic, or a wheat/barley addition line, amongst others, the only requirement that the genomic DNA is at least about 80% identical to the genome of a wheat plant as determined by standard DNA melting curve analyses.

The term "starch synthase II" or "wSSII" or similar term shall be taken to refer to a starch synthase as hereinbefore defined that is detectable in the starch granule of a plant seed endosperm and possesses one or more properties selected from the group consisting of:

(i) it is immunologically cross-reactive with the wheat starch granule proteins designated Sgp-B1 and/or Sgp-D1 and/or Sgp-A1, having estimated molecular weights of about 85 kDa to about 115 kDa;

(ii) it is encoded by one of a homeologous set of genes localised on wheat chromosomes 7B or 7A or 7D;

(iii) it is encoded by a nucleotide sequence that comprises at least about 15 nucleotides in length derived from any one or more of SEQ ID NOS: 1, 3, 5, or 37 or a complementary nucleotide sequence thereto;

(iv) it is encoded by a nucleotide sequence that is at least about 85% identical to one or more of the nucleotide sequences set forth in SEQ ID NOS: 1, 3, 5, or 37, or a complementary nucleotide sequence thereto;

(v) it comprises an amino acid sequence having at least about 85% identity to one or more of SEQ ID NOS: 2 or 4 or 6;

(vi) it comprises at least about 5 contiguous amino acids, preferably at least about 10 contiguous amino acids, more preferably at least about 15 contiguous amino acids, even more preferably at least about 20 contiguous amino acids and still even more preferably at least about 25-50 contiguous amino acids of the amino acid sequences set forth in SEQ ID NOS: 2 or 4 or 6;

(vii) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

(a) KVGGLGDVVTS;

(b) GHTVEVILPKY;

(c) HDWSSAPVAWLYKEHY;

(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVVLLGSA;

(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS; and

(h)TGGLVDTV,

in addition to any one or more of (i) to (vi); and

(viii) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

(a) KTGGLGDVAGA;

(b) GHRVMVVVPRY;

(c) NDWHTALLPVYLKAYY;

(d) GIVNGIDNMEWNPEVD;

(e) DVPLLGFIGRLDGQKG;

(f) DVQLVMLGTG;

(g)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(h)VGG(V/L)RDTV,

in addition to any one or more of (i) to (vi).

The term "starch synthase III" or "wSSIII" or similar term shall be taken to refer to a starch synthase as hereinbefore defined that possesses one or more properties selected from the group consisting of:

(i) it is encoded by a nucleotide sequence that comprises at least about 15 nucleotides in length derived from any one or more of SEQ ID NOS: 7, 9, 11-16, or 38, or a complementary nucleotide sequence thereto;

(ii) it is encoded by a nucleotide sequence that is at least about 85% identical to one or more of the nucleotide sequences set forth in SEQ ID NOS: 7, 9, 11-16, or 38, or a complementary nucleotide sequence thereto; and

(iii) it comprises an amino acid sequence having at least about 85% identity to one or more of SEQ ID NOS: 8 or 10;

(iv) it comprises at least about 5 contiguous amino acids, preferably at least about 10 contiguous amino acids, more preferably at least about 15 contiguous amino acids, even more preferably at least about 20 contiguous amino acids and still even more preferably at least about 25-50 contiguous amino acids of the amino acid sequences set forth in SEQ ID NOS: 8 or 10;

(v) which comprises a conserved amino acid sequence having at least 25%

identity to an amino acid sequence selected from the group consisting of:

(a) KVGGLGDVVTS;

(b) GHTVEVILPKY;

(c) HDWSSAPVAWLYKEHY;

(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVVLLGSA;

(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS; and

(h)TGGLVDTV

in addition to any one or more of (i) to (iv); and

(vi) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

(a) KTGGLGDVAGA;

(b) GHRVMVVVPRY;

(c) NDWHTALLPVYLKAYY;

(d) GIVNGIDNMEWNPEVD;

(e) DVPLLGFIGRLDGQKG;

(f) DVQLVMLGTG;

(g)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(h)VGG(V/L)RDTV,

in addition to any one or more of (i) to (iv).

In a more preferred embodiment, the WSSII or WSSIII polypeptide encoded by the nucleic acid molecule of the present invention will comprise a substantial contiguous region of any one of SEQ ID NOS: 2, 4, 6, 8 or 10 or 17 sufficient to possess the biological activity of a starch synthase polypeptide.

For the purposes of nomenclature, the nucleotide sequence set forth in SEQ ID NO: 1 relates to the cDNA molecule encoding the WSSII (i.e. Sgp-B1) polypeptide of wheat. The amino acid sequence of the corresponding polypeptide is set forth herein as SEQ ID NO:2. The nucleotide sequence set forth in SEQ ID NO: 3 relates to the

cDNA molecule encoding the WSSII (i.e. Sgp-A1) polypeptide of wheat. The amino acid sequence of the corresponding polypeptide is set forth herein as SEQ ID NO:4. The nucleotide sequence set forth in SEQ ID NO: 5 relates to the cDNA molecule encoding the WSSII (i.e. Sgp-D1) polypeptide of wheat. The amino acid sequence of the corresponding polypeptide is set forth herein as SEQ ID NO:6. The nucleotide sequences set forth in SEQ ID NOs: 7 and 9 relate, respectively, to full-length and partial cDNA molecules encoding the WSSIII polypeptide of wheat. The amino acid sequences of the corresponding polypeptides are set forth herein as SEQ ID NOS: 8 and 10, respectively. The nucleotide sequences set forth in SEQ ID NOs: 11 to 16 relates to fragments of the genomic gene encoding the WSSIII polypeptide of wheat, significant protein-encoding regions of which are described by reference to Table 4 and Figure 11. The nucleotide sequence set forth in SEQ ID NO: 37 relates to the WSSII genomic gene of *Triticum tauschii*, corresponding to the WSSII gene of the D-genome of wheat, which encodes the WSSIII polypeptide. The nucleotide sequence set forth in SEQ ID NO: 38 relates to the wheat WSSIII genomic gene.

Preferably, the isolated nucleic acid molecule of the present invention comprises a sequence of nucleotides which encodes, or is complementary to a nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8, or 10 and more preferably, which additionally comprises which comprises one or more amino acid sequences selected from the group consisting of:

(a) KVGGLGDVVTS;

(b) GHTVEVILPKY;

(c) HDWSSAPVAWLYKEHY;

(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVVLLGSA;

(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS;

(h)TGGLVDTV;
(i) KTGGLGDVAGA;
(j) GHRVMVVVPRY;
(k) NDWHTALLPVYLKAYY;
(l) GIVNGIDNMEWNPEVD;
(m) DVPLLGFIGRLDGQKG;
(n) DVQLVMLGTG;
(o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
(p)VGG(V/L)RDTV.

The present invention clearly extends to homologues, analogues and derivatives of the wheat starch synthase II and III genes exemplified by the nucleotide sequences set forth herein as SEQ ID NOs: 1, 3, 5, 7, 9,11-16, 37 or 38.

Preferred starch synthase genes may be derived from a naturally-occurring starch synthase gene by standard recombinant techniques. Generally, a starch synthase gene may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions. Nucleotide insertional derivatives of the starch synthase gene of the present invention include 5′ and 3′ terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more nucleotides from the sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by the codon. Alternatively, substituents are designed to alter one amino acid for another similar acting amino acid, or amino acid of like charge, polarity, or hydrophobicity.

For the present purpose, "homologues" of a nucleotide sequence shall be taken to

refer to an isolated nucleic acid molecule which is substantially the same as the nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence within said sequence, of one or more nucleotide substitutions, insertions, deletions, or rearrangements.

"Analogues" of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as a nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence of any non-nucleotide constituents not normally present in said isolated nucleic acid molecule, for example carbohydrates, radiochemicals including radionucleotides, reporter molecules such as, but not limited to DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

"Derivatives" of a nucleotide sequence set forth herein shall be taken to refer to any isolated nucleic acid molecule which contains significant sequence similarity to said sequence or a part thereof. Generally, the nucleotide sequence of the present invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives of the nucleotide sequence of the present invention include 5′ and 3′ terminal fusions as well as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues. Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence of said sequence, although random insertion is also possible with suitable screening of the resulting product being performed. Deletional variants are characterised by the removal of one or more nucleotides from the nucleotide sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place.

The present invention extends to the isolated nucleic acid molecule when integrated into the genome of a cell as an addition to the endogenous cellular complement of starch synthase genes, irrespective of whether or not the introduced nucleotide

sequence is translatable or non-translatable to produce a polypeptide. The present invention clearly contemplates the introduction of additional copies of starch synthase genes into plants, particularly wheat plants, in the antisense orientation to reduce the expression of particular wheat starch synthase genes. As will be known to those skilled in the art, such antisense genes are non-translatable, notwithstanding that they can be expressed to produce antisense mRNA molecules.

The said integrated nucleic acid molecule may, or may not, contain promoter sequences to regulate expression of the subject genetic sequence.

Accordingly, the present invention clearly encompasses preferred homologues, analogues and derivatives that comprise a sequence of nucleotides which encodes, or is complementary to a nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof selected from the following:

(i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;

(ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;

(iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

(a) KVGGLGDVVTS;

(b) GHTVEVILPKY;

(c) HDWSSAPVAWLYKEHY;

(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVVLLGSA;

(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS; and

(h)TGGLVDTV

and wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10; and

(iv) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

(a) KTGGLGDVAGA;

(b) GHRVMVVVPRY;

(c) NDWHTALLPVYLKAYY;

(d) GIVNGIDNMEWNPEVD;

(e) DVPLLGFIGRLDGQKG;

(f) DVQLVMLGTG;

(g)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(h)VGG(V/L)RDTV,

and wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10.

Preferably, the isolated nucleic acid molecule encodes a starch synthase polypeptide, protein or enzyme that comprises two, more preferably three, more preferably four, more preferably five, more preferably six, more preferably seven and even more preferably eight of the conserved amino acid motifs listed *supra*. Even more preferably, the said amino acid motifs are located in a relative configuration such as that shown for the wheat SSII or wheat SSIII polypeptides described herein.

In a preferred embodiment, the isolated nucleic acid molecule encodes a starch

synthase polypeptide, protein or enzyme having at least about 90% amino acid sequence identity to any one of SEQ ID NOS: 2, 4, 6, 8 or 10, more preferably having at least about 95% or about 97% or about 99% identity to any one of said amino acid sequences.

In an alternative embodiment, the present invention provides an isolated nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof, wherein said nucleic acid molecule comprises a nucleotide sequence having at least about 85% nucleotide sequence identity to any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38, or a degenerate nucleotide sequence thereto or a complementary nucleotide sequence thereto.

By "degenerate nucleotide sequence" is meant a nucleotide sequence that encodes a substantially identical amino acid sequence as a stated nucleotide sequence.

In a preferred embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38, or is at least about 90% identical, more preferably at least about 95% or 97% or 99% identical to all or a protein-encoding part thereof.

In an alternative embodiment, preferred homologues, analogues and derivatives of the nucleic acid molecule of the present invention encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof and comprises a nucleotide sequence that is capable of hybridising under at least moderate stringency hybridisation conditions to at least about 30 contiguous nucleotides derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38, or a complementary nucleotide sequence thereto.

For the purposes of defining the level of stringency, a low stringency is defined herein as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. Generally, the stringency is increased by reducing the concentration of SSC

buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. A moderate stringency comprises a hybridisation and/or a wash carried out in 0.2 x SSC-2 x SSC buffer, 0.1% (w/v) SDS at 42°C to 65°C, while a high stringency comprises a hybridisation and/or a wash carried out in 0.1xSSC-0.2 x SSC buffer, 0.1% (w/v) SDS at a temperature of at least 55°C. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of further clarification only, reference to the parameters affecting hybridisation between nucleic acid molecules is found in pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

Those skilled in the art will be aware of procedures for the isolation of further wheat starch synthase genes to those specifically described herein or homologues, analogues or derivatives of said genes, for example further cDNA sequences and genomic gene equivalents, when provided with one or more of the nucleotide sequences set forth in SEQ ID NOs: 1, 3, 5, 7, 9,11-16, 37, or 38. In particular, amplifications and/or hybridisations may be performed using one or more nucleic acid primers or hybridisation probes comprising at least 10 contiguous nucleotides and preferably at least about 20 contiguous nucleotides or 50 contiguous nucleotides derived from the nucleotide sequences set forth herein, to isolate cDNA clones, mRNA molecules, genomic clones from a genomic library (in particular genomic clones containing the entire 5' upstream region of the gene including the promoter sequence, and the entire coding region and 3′-untranslated sequences), and/or synthetic oligonucleotide molecules, amongst others. The present invention clearly extends to such related sequences.

Accordingly, a second aspect of the present invention provides a method of isolating a nucleic acid molecule that encodes a starch synthase polypeptide, protein or enzyme said method comprising:

(i) hybridising a probe or primer comprising at least about 15 contiguous nucleotides in length derived from any one of SEQ ID NOS 1, 3, 5, 7, 9,11-16, 37, or 38, or a complementary nucleotide sequence thereto to single-stranded or double-stranded mRNA, cDNA or genomic DNA; and

(ii) detecting the hybridised mRNA, cDNA or genomic DNA using a detecting means.

Preferably, the detecting means is a reporter molecule covalently attached to the probe or primer molecule or alternatively, a polymerase chain reaction format.

An alternative method contemplated in the present invention involves hybridising two nucleic acid "primer molecules" to a nucleic acid "template molecule" which comprises a related starch synthase gene or related starch synthase genetic sequence or a functional part thereof, wherein the first of said primers comprises contiguous nucleotides derived from any one or more of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38, and the second of said primers comprises contiguous nucleotides complementary to any one or more of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38. Specific nucleic acid molecule copies of the template molecule are amplified enzymatically in a polymerase chain reaction, a technique that is well known to one skilled in the art.

In a preferred embodiment, each nucleic acid primer molecule is at least 10 nucleotides in length, more preferably at least 20 nucleotides in length, even more preferably at least 30 nucleotides in length, still more preferably at least 40 nucleotides in length and even still more preferably at least 50 nucleotides in length.

Furthermore, the nucleic acid primer molecules consists of a combination of any of the nucleotides adenine, cytidine, guanine, thymidine, or inosine, or functional analogues or derivatives thereof which are at least capable of being incorporated into a polynucleotide molecule without having an inhibitory effect on the hybridisation of said primer to the template molecule in the environment in which it is used.

Furthermore, one or both of the nucleic acid primer molecules may be contained in an aqueous mixture of other nucleic acid primer molecules, for example a mixture of degenerate primer sequences which vary from each other by one or more nucleotide substitutions or deletions. Alternatively, one or both of the nucleic acid primer molecules may be in a substantially pure form.

The nucleic acid template molecule may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell, or a plant cell. Preferably, the nucleic acid template molecule is derived from a plant cell, tissue or organ, in particular a cell, tissue or organ derived from a wheat or barley plant or a progenitor species, or a relative thereto such as the diploid *Triticum tauschii* or other diploid, tetraploid, aneuploid, polyploid, nullisomic, or a wheat/barley addition line, amongst others.

Those skilled in the art will be aware that there are many known variations of the basic polymerase chain reaction procedure, which may be employed to isolate a related starch synthase gene or related starch synthase genetic sequence when provided with the nucleotide sequences set forth herein. Such variations are discussed, for example, in McPherson *et al* (1991). The present invention extends to the use of all such variations in the isolation of related starch synthase genes or related starch synthase genetic sequences using the nucleotide sequences embodied by the present invention.

As exemplified herein, the present inventors have isolated several wheat starch synthase genes using both hybridisation and polymerase chain reaction approaches, employing novel probes and primer sequences to do so.

Accordingly, a third aspect of the invention provides an isolated probe or primer comprising at least about 15 contiguous nucleotides in length derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38, or a complementary nucleotide sequence thereto.

Preferably, the probe or primer comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 25 to 34.

The isolated nucleic acid molecule of the present invention may be introduced into and expressed in any cell, for example a plant cell, fungal cell, insect cell. animal cell, yeast cell or bacterial cell. Those skilled in the art will be aware of any modifications which are required to the codon usage or promoter sequences or other regulatory

sequences, in order for expression to occur in such cells.

A further aspect of the invention provides a method of assaying for the presence or absence of a starch synthase isoenzyme or the copy number of a gene encoding same in a plant, comprising contacting a biological sample derived from said plant with an isolated nucleic acid molecule derived from any one of SEQ ID NOS 1, 3, 5, 7, 9,11-16, 37, or 38, or any one of SEQ ID NOS: 25 to 34, or a complementary nucleotide sequence thereto for a time and under conditions sufficient for hybridisation to occur and then detecting said hybridisation using a detection means.

The detection means according to this aspect of the invention is any nucleic acid based hybridisation or amplification reaction.

The hexaploid nature of wheat prevents the straightforward identification of starch synthase allelic variants by hybridisation using the complete starch synthase-encoding sequence, because the similarities between the various alleles generally results in significant cross-hybridisation. Accordingly, sequence-specific hybridisation probes are required to distinguish between the various alleles. Similarly, wherein PCR is used to amplify specific allelic variants of a starch synthase gene, one or more sequence-specific amplification primers are generally required. As will be apparent from the amino acid sequence comparisons provided herein, such as in Figures 3 and 13, non-conserved regions of particular wheat starch synthase polypeptides are particularly useful for the design of probes and primers that are capable of distinguishing between one or more starch synthase polypeptide isoenzyme or allelic variant. The present invention clearly contemplates the design of such probes and primers based upon the sequence comparisons provided herein.

In the performance of this embodiment of the present invention, the present inventors particularly contemplate the identification of wheat starch synthase null alleles or alternatively, mutations wherein specific amino acids are inserted or deleted or substituted, compared to one or more of the wheat SSII or SSIII alleles disclosed

herein. Such null alleles and other allelic variants are readily identifiable using PCR screening which employs amplification primers based upon the nucleotide and amino acid sequences disclosed herein for SSII and/or SSIII. Once identified, the various mutations can be stacked or pyramided into one or more new wheat lines, such as by introgression and/or standard plant breeding and/or recombinant approaches (eg. transformation, transfection, etc) thereby producing a novel germplasm which exhibits altered starch properties compared to existing lines. DNA markers based upon the nucleotide and amino acid sequences disclosed herein for SSII and/or SSIII can be employed to monitor the stacking of genes into the new lines and to correlate the presence of particular genes with starch phenotypes of said lines.

In this regard, a significant advantage conferred by the present invention is the design of new DNA markers that reveal polymorphisms such as, for example, length polymorphisms, restriction site polymorphisms, and single nucleotide polymorphisms, amongst others, between wheat starch synthases and, in particular, between wheat GBSS and/or SSI and/or SSII and/or SSIII, or between allelic variants of one or more of said starch synthases, that can be used to identify the three genomes of hexaploid wheats (i.e., the A, B and D genomes).

Preferably, such DNA markers are derived from the intron region of a starch synthase gene disclosed herein, more preferably the wheat SSII and/or the wheat SSIII gene. Those skilled in the art will be aware that such regions generally have a higher degree of variation than in the protein-encoding regions and, as a consequence, are particularly useful in identifying specific allelic variants of a particular gene, such as allelic variants contained in any one of the three wheat genomes, or alternatively or in addition, for the purpose of distinguishing between wheat GBSS, SSI, SSII or SSIII genes.

A further approach contemplated by the present inventors is the design of unique isoenzyme-specific and/or allele-specific peptides based upon the amino acid sequence disclosed herein as SEQ ID NOS: 25 and/or SEQ ID NO: 4 and/or SEQ ID

NO: 6 and/or SEQ ID NO: 8 and/or SEQ ID NO: 10, which peptides are then used to produce polyclonal or monoclonal antibodies by conventional means. Alternatively, the genes encoding these polypeptides or unique peptide regions thereof can be introduced in an expressible format into an appropriate prokaryotic or eukaryotic expression system, where they can be expressed to produce the isoenzyme-specific and/or allele-specific peptides for antibody production. Such antibodies may also be used as markers for the purpose of both identifying parental lines and germplasms and monitoring the stacking of genes in new lines, using conventional immunoassays such as, for example, ELISA and western blotting.

A further aspect of the present invention utilises the above-mentioned nucleic acid based assay method in the breeding and/or selection of plants which express or do not express particular starch synthase isoenzymes or alternatively, which express a particular starch synthase isoenzyme at a particular level in one or more plant tissues. This aspect clearly extends to the selection of transformed plant material which contains one or more of the isolated nucleic acid molecules of the present invention.

Yet another aspect of the present invention provides for the expression of the nucleic acid molecule of the present invention in a suitable host (e.g. a prokaryote or eukaryote) to produce full length or non-full length recombinant starch synthase gene products.

Hereinafter the term "starch synthase gene product" shall be taken to refer to a recombinant product of a starch synthase gene of the present invention.

Preferably, the recombinant starch synthase gene product comprises an amino acid sequence having the catalytic activity of a starch synthase polypeptide or a functional mutant, derivative part, fragment, or analogue thereof.

In a particularly preferred embodiment of the invention, the recombinant starch synthase gene product is selected from the following:

(i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;

(ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10; and

(iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

(a) KVGGLGDVVTS;

(b) GHTVEVILPKY;

(c) HDWSSAPVAWLYKEHY;

(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVVLLGSA;

(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS;

(h)TGGLVDTV; (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;

(ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;

(iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at

least 25% identity to an amino acid sequence selected from the group consisting of:

(a) KVGGLGDVVTS;
(b) GHTVEVILPKY;
(c) HDWSSAPVAWLYKEHY;
(d) GILNGIDPDIWDPYTD;
(e) DVPIVGIITRLTAQKG;
(f) NGQVVLLGSA;
(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS; and
(h)TGGLVDTV;
(i) KTGGLGDVAGA;
(j) GHRVMVVVPRY;
(k) NDWHTALLPVYLKAYY;
(l) GIVNGIDNMEWNPEVD;
(m) DVPLLGFIGRLDGQKG;
(n) DVQLVMLGTG;
(o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
(p)VGG(V/L)RDTV.

Accordingly, the present invention clearly extends to homologues, analogues and derivatives of the amino acid sequences set forth herein as SEQ ID NOS: 2, 4, 6, 8 and 10.

In the present context, "homologues" of an amino acid sequence refer to those polypeptides, enzymes or proteins which have a similar catalytic activity to the amino acid sequences described herein, notwithstanding any amino acid substitutions, additions or deletions thereto. A homologue may be isolated or derived from the same or another plant species as the species from which the polypeptides of the invention are derived.

"Analogues" encompass polypeptides of the invention notwithstanding the occurrence

of any non-naturally occurring amino acid analogues therein.

"Derivatives" include modified peptides in which ligands are attached to one or more of the amino acid residues contained therein, such as carbohydrates, enzymes, proteins, polypeptides or reporter molecules such as radionuclides or fluorescent compounds. Glycosylated, fluorescent, acylated or alkylated forms of the subject peptides are particularly contemplated by the present invention. Additionally, derivatives of an amino acid sequence described herein which comprises fragments or parts of the subject amino acid sequences are within the scope of the invention, as are homopolymers or heteropolymers comprising two or more copies of the subject polypeptides. Procedures for derivatizing peptides are well-known in the art.

Substitutions encompass amino acid alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which an amino acid residue contained in a starch synthase gene product is replaced with another naturally-occurring amino acid of similar character, for example Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln or Phe↔Trp↔Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a starch synthase gene product described herein is substituted with an amino acid with different properties, such as a naturally-occurring amino acid from a different group (eg. substituted a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid.

Non-conventional amino acids encompassed by the invention include, but are not limited to those listed in Table 2.

Amino acid substitutions are typically of single residues, but may be of multiple residues, either clustered or dispersed.

Amino acid deletions will usually be of the order of about 1-10 amino acid residues, while insertions may be of any length. Deletions and insertions may be made to the N-terminus, the C-terminus or be internal deletions or insertions. Generally, insertions within the amino acid sequence will be smaller than amino- or carboxy-terminal fusions and of the order of 1-4 amino acid residues.

A homologue, analogue or derivative of a starch synthase gene product as referred to herein may readily be made using peptide synthetic techniques well-known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. Techniques for making substituent mutations at pre-determined sites using recombinant DNA technology, for example by M13 mutagenesis, are also well-known. The manipulation of nucleic acid molecules to produce variant peptides, polypeptides or proteins which manifest as substitutions, insertions or deletions are well-known in the art.

The starch synthase gene products described herein may be derivatized further by the inclusion or attachment thereto of a protective group which prevents, inhibits or slows proteolytic or cellular degradative processes. Such derivatization may be useful where the half-life of the subject polypeptide is required to be extended, for example to increase the amount of starch produced in the endosperm or alternatively, to increase the amount of protein produced in a bacterial or eukaryotic expression system. Examples of chemical groups suitable for this purpose include, but are not limited to, any of the non-conventional amino acid residues listed in Table 2, in particular a D-stereoisomer or a methylated form of a naturally-occurring amino acid listed in Table 1. Additional chemical groups which are useful for this purpose are selected from the list comprising aryl or heterocyclic N-acyl substituents, polyalkylene oxide moieties, desulphatohirudin muteins, alpha-muteins, alpha-aminophosphonic acids, water-soluble polymer groups such as polyethylene glycol attached to sugar residues using hydrazone or oxime groups, benzodiazepine dione derivatives, glycosyl groups such as beta-glycosylamine or a derivative thereof, isocyanate conjugated to a polyol functional group or polyoxyethylene polyol capped with diisocyanate, amongst others. Similarly, a starch synthase gene product or a homologue, analogue or derivative

thereof may be cross-linked or fused to itself or to a protease inhibitor peptide, to reduce susceptibility of said molecule to proteolysis.

In a particularly preferred embodiment, the percentage similarity to in any one of SEQ ID NOS: 2, 4, 6, 8 or 10 is at least about 90%, more preferably at least about 95%, even more preferably at least about 97% and even more preferably at least about 98%, or about 99% or 100%.

In a related embodiment, the present invention provides a "sequencably pure" form of the amino acid sequence described herein. "Sequencably pure" is hereinbefore described as substantially homogeneous to facilitate amino acid determination.

In a further related embodiment, the present invention provides a "substantially homogeneous" form of the subject amino acid sequence, wherein the term "substantially homogeneous" is hereinbefore defined as being in a form suitable for interaction with an immunologically interactive molecule. Preferably, the polypeptide is at least 20% homogeneous, more preferably at least 50% homogeneous, still more preferably at least 75% homogeneous and yet still more preferably at least about 95-100% homogenous, in terms of activity per microgram of total protein in the protein preparation.

To produce the recombinant polypeptide of the present invention, the coding region of a starch synthase gene described herein or a functional homologue, analogue or derivative thereof is placed operably in connection with a promoter sequence in the sense orientation, such that a starch synthase gene product is capable of being expressed under the control of said promoter sequence.

In the present context, the term "in operable connection with" means that expression of the isolated nucleotide sequence is under the control of the promoter sequence with which it is connected, regardless of the relative physical distance of the sequences from each other or their relative orientation with respect to each other.

Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. A promoter is usually, but not necessarily, positioned upstream or 5′, of a structural gene, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene.

In the present context, the term "promoter" is also used to describe a synthetic or fusion molecule, or derivative which confers, activates or enhances expression of a structural gene or other nucleic acid molecule, particularly in a plant cell and more preferably in a wheat plant or other monocotyledonous plant cell, tissue or organ. Preferred promoters may contain additional copies of one or more specific regulatory elements, to further enhance expression and/or to alter the spatial expression and/or temporal expression. For example, regulatory elements which confer copper inducibility may be placed adjacent to a heterologous promoter sequence, thereby conferring copper inducibility on the expression of said molecule.

Those skilled in the art will be aware that in order to obtain optimum expression of the starch synthase gene of the present invention, it is necessary to position said gene in an appropriate configuration such that expression is controlled by the promoter sequence. Promoters are generally positioned 5′ (upstream) to the genes that they control. In the construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the genes from

which it is derived. Again, as is known in the art, some variation in this distance can also occur.

Examples of promoters suitable for expressing the starch synthase gene of the present invention include viral, fungal, bacterial, animal and plant derived promoters capable of functioning in prokaryotic or eukaryotic cells. Preferred promoters are those capable of regulating the expression of the subject starch synthase genes in plants cells, fungal cells, insect cells, yeast cells, animal cells or bacterial cells, amongst others. Particularly preferred promoters are capable of regulating expression of the subject nucleic acid molecules in monocotyledonous plant cells. The promoter may regulate the expression of the said molecule constitutively, or differentially with respect to the tissue in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, or plant pathogens, or metal ions, amongst others.

Accordingly, strong constitutive promoters are particularly preferred for the purposes of the present invention.

Examples of preferred promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, *lac* operator-promoter, *tac* promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, CaMV 35S promoter, SCSV promoter, SCBV promoter and the like.

Particularly preferred promoters operable in plant cells include, for example the CaMV 35S promoter, and the SCBV promoter. Those skilled in the art will readily be aware of additional promoter sequences other than those specifically described.

In a particularly preferred embodiment, the promoter may be derived from a genomic starch synthase gene. Preferably, the promoter sequence comprises nucleotide sequences that are linked *in vivo* to nucleotide sequences set forth in any one of SEQ ID NOs: 1, 3, 5, 7, 9,11-16, 37, or 38. By "linked *in vivo*" means that the promoter is present in its native state in the genome of a wheat plant where it controls expression

of the starch synthase gene of the present invention.

Conveniently, genetic constructs are employed to facilitate expression of a starch synthase genetic sequence of the present invention or a functional derivative, part, homologue, or analogue thereof. To produce a genetic construct, the starch synthase gene of the invention is inserted into a suitable vector or episome molecule, such as a bacteriophage vector, viral vector or a plasmid, cosmid or artificial chromosome vector which is capable of being maintained and/or replicated and/or expressed in the host cell, tissue or organ into which it is subsequently introduced. The said genetic construct comprises the subject nucleic acid molecule placed operably under the control of a promoter sequence and optionally, a terminator sequence.

The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3'-end of a primary transcript. Terminators active in bacteria, yeasts, animal cells and plant cells are known and described in the literature. They may be isolated from bacteria, fungi, viruses, animals and/or plants.

Examples of terminators particularly suitable for use in expressing the nucleic acid molecule of the present invention in plant cells include the nopaline synthase (NOS) gene terminator of *Agrobacterium tumefaciens,* the terminator of the Cauliflower mosaic virus (CaMV) 35S gene, and the *zein* gene terminator from *Zea mays.*

Genetic constructs will generally further comprise one or more origins of replication and/or selectable marker gene sequences.

The origin of replication can be functional in a bacterial cell and comprise, for example, the pUC or the ColE1 origin. Alternatively, the origin of replication is operable in a eukaryotic cell, tissue and more preferably comprises the 2 micron (2μm) origin of replication or the SV40 origin of replication.

As used herein, the term "selectable marker gene" includes any gene which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a genetic construct of the invention or a derivative thereof.

Suitable selectable marker genes contemplated herein include the ampicillin-resistance gene (Amp^r), tetracycline-resistance gene (Tc^r), bacterial kanamycin-resistance gene (Kan^r), is the zeocin resistance gene (Zeocin is a drug of bleomycin family which is trademark of InVitrogen Corporation), the *AURI-C* gene which confers resistance to the antibiotic aureobasidin A, phosphinothricin-resistance gene, neomycin phosphotransferase gene (*nptII*), hygromycin-resistance gene, β-glucuronidase (GUS) gene, chloramphenicol acetyltransferase (CAT) gene, green fluorescent protein-encoding gene or the luciferase gene, amongst others. Those skilled in the art will be aware of other selectable marker genes useful in the performance of the present invention and the subject invention is not limited by the nature of the selectable marker gene.

Usually, an origin of replication or a selectable marker gene suitable for use in bacteria is physically-separated from those genetic sequences contained in the genetic construct which are intended to be expressed or transferred to a eukaryotic cell, or integrated into the genome of a eukaryotic cell.

Standard methods can be used to introduce genetic constructs into a cell, tissue or organ for the purposes of modulating gene expression. Particularly preferred methods suited to the introduction of synthetic genes and genetic constructs comprising same to eukaryotic cells include liposome-mediated transfection or transformation, transformation of cells with attenuated virus particles or bacterial cells and standard procedures for the transformation of plant and animal cells, tissues, organs or organisms. Any standard means may be used for their introduction including cell mating, transformation or transfection procedures known to those skilled in the art or described by Ausubel *et al.* (1992).

In a further embodiment of the present invention, the starch synthase genes of the present invention and genetic constructs comprising same are adapted for integration into the genome of a cell in which it is expressed. Those skilled in the art will be aware that, in order to achieve integration of a genetic sequence or genetic construct into the genome of a host cell, certain additional genetic sequences may be required. In the case of plants, left and right border sequences from the T-DNA of the *Agrobacterium tumefaciens* Ti plasmid will generally be required.

The invention further contemplates increased starch and/or modified starch composition in transgenic plants expressing the nucleic acid molecule of the invention in the sense orientation such that the activity of one or more starch synthase isoenzymes is increased therein. By increasing the level of one or more starch synthase isoenzymes, the deposition of starch in the amyloplast or chloroplast is increased and/or a modified starch granule structure is produced and/or starch composition is modified and/or the amylose/amylopectin ratio is altered in the plant.

Wherein it is desired to increase the synthesis of a particular starch synthase isoenzyme in a plant cell, the coding region of a starch synthase gene is placed operably behind a promoter, in the sense orientation, such that said starch synthase is expressed under the control of said promoter sequence. In a preferred embodiment, the starch synthase genetic sequence is a starch synthase genomic sequence, cDNA molecule or protein-coding sequence.

Wherein it is desirable to reduce the level of a particular starch synthase isoenzyme in a plant cell, the nucleic acid molecule of the present invention can be expressed in the antisense orientation, as an antisense molecule or a ribozyme molecule, under the control of a suitable promoter.

Alternatively, the nucleic acid molecule of the present invention may also be expressed in the sense orientation, in the form of a co-suppression molecule, to reduce the level of a particular starch synthase isoenzyme in a plant cell. As will be known to those skilled in the art, co-suppression molecules that comprise inverted repeat sequences

of a target nucleic acid molecule provide optimum efficiency at reducing expression of said target nucleic acid molecule and, as a consequence, the present invention clearly contemplates the use of inverted repeat sequences of any one or more of the starch synthase genetic sequences exemplified herein, or inverted repeat sequences of a homologue, analogue or derivative of said starch synthase genetic sequences, to reduce the level of a starch synthase isoenzyme in a plant.

The expression of an antisense, ribozyme or co-suppression molecule comprising a starch synthase gene in a cell such as a plant cell, fungal cell, insect cell, animal cell, yeast cell or bacterial cell, may also increase the availability of carbon as a precursor for a secondary metabolite other than starch (e.g. sucrose or cellulose). By targeting the endogenous starch synthase gene, expression is diminished, reduced or otherwise lowered to a level that results in reduced deposition of starch in the amyloplast or chloroplast and/or leads to modified starch granule structure and/or composition and/or altered amylose/amylopectin ratio.

Accordingly, a further aspect of the present invention provides a method of modifying the starch content and/or starch composition of one or more tissues or organs of a plant, comprising expressing therein a sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38, or a complementary nucleotide sequence thereto for a time and under conditions sufficient for the enzyme activity of one or more starch synthase isoenzymes to be modified. This aspect of the invention clearly extends to the introduction of the sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule to isolated plant cells, tissues or organs or organelles by cell fusion or transgenic means and the regeneration of intact plants therefrom.

Co-suppression is the reduction in expression of an endogenous gene that occurs when one or more copies of said gene, or one or more copies of a substantially similar

gene are introduced into the cell, preferably in the form of an inverted repeat structure.

The present inventors have discovered that the genetic sequences disclosed herein are capable of being used to modify the level of starch when expressed, particularly when expressed in plants cells. Accordingly, the present invention clearly extends to the modification of starch biosynthesis in plants, in particular wheat or barley plants or a progenitor plant species, or a relative thereto such as the diploid *Triticum tauschii* or other diploid, tetraploid, aneuploid, polyploid, nullisomic, or a wheat/barley addition line, amongst others.

In particular, the present invention contemplates decreased starch production and/or modified starch composition in transgenic plants expressing the nucleic acid molecule of the invention in the antisense orientation or alteratively, expressing a ribozyme or co-suppression molecule comprising the nucleic acid sequence of the invention such that the activity of one or more starch synthase isoenzymes is decreased therein.

In the context of the present invention, an antisense molecule is an RNA molecule which is transcribed from the complementary strand of a nuclear gene to that which is normally transcribed to produce a "sense" mRNA molecule capable of being translated into a starch synthase polypeptide. The antisense molecule is therefore complementary to the mRNA transcribed from a sense starch synthase gene or a part thereof. Although not limiting the mode of action of the antisense molecules of the present invention to any specific mechanism, the antisense RNA molecule possesses the capacity to form a double-stranded mRNA by base pairing with the sense mRNA, which may prevent translation of the sense mRNA and subsequent synthesis of a polypeptide gene product.

Ribozymes are synthetic RNA molecules which comprise a hybridising region complementary to two regions, each of at least 5 contiguous nucleotide bases in the target sense mRNA. In addition, ribozymes possess highly specific endoribonuclease

activity, which autocatalytically cleaves the target sense mRNA. A complete description of the function of ribozymes is presented by Haseloff and Gerlach (1988) and contained in International Patent Application No. WO89/05852.

The present invention extends to ribozyme which target a sense mRNA encoding a native starch synthase gene product, thereby hybridising to said sense mRNA and cleaving it, such that it is no longer capable of being translated to synthesise a functional polypeptide product.

According to this embodiment, the present invention provides a ribozyme or antisense molecule comprising at least 5 contiguous nucleotide bases derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38, or a complementary nucleotide sequence thereto or a homologue, analogue or derivative thereof, wherein said antisense or ribozyme molecule is able to form a hydrogen-bonded complex with a sense mRNA encoding a starch synthase gene product to reduce translation thereof.

In a preferred embodiment, the antisense or ribozyme molecule comprises at least 10 to 20 contiguous nucleotides derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38, or a complementary nucleotide sequence thereto or a homologue, analogue or derivative thereof. Although the preferred antisense and/or ribozyme molecules hybridise to at least about 10 to 20 nucleotides of the target molecule, the present invention extends to molecules capable of hybridising to at least about 50-100 nucleotide bases in length, or a molecule capable of hybridising to a full-length or substantially full-length mRNA encoded by a starch synthase gene.

Those skilled in the art will be aware of the necessary conditions, if any, for selecting or preparing the antisense or ribozyme molecules of the invention.

It is understood in the art that certain modifications, including nucleotide substitutions amongst others, may be made to the antisense and/or ribozyme molecules of the present invention, without destroying the efficacy of said molecules in inhibiting the expression of a starch synthase gene. It is therefore within the scope of the present

invention to include any nucleotide sequence variants, homologues, analogues, or fragments of the said gene encoding same, the only requirement being that said nucleotide sequence variant, when transcribed, produces an antisense and/or ribozyme molecule which is capable of hybridising to a sense mRNA molecule which encodes a starch synthase gene product.

Gene targeting is the replacement of an endogenous gene sequence within a cell by a related DNA sequence to which it hybridises, thereby altering the form and/or function of the endogenous gene and the subsequent phenotype of the cell. According to this embodiment, at least a part of the DNA sequence defined by any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38 may be introduced into target cells containing an endogenous gene that encodes a particular starch synthase isoenzyme, thereby replacing said endogenous gene. According to this embodiment, the polypeptide product of the gene targetting molecule generally encodes a starch synthase isoenzyme that possesses different catalytic activity to the polypeptide product of the endogenous gene, producing in tum modified starch content and/or composition in the target cell.

The present invention extends to genetic constructs designed to facilitate expression of a sense molecule, an antisense molecule, ribozyme molecule, co-suppression molecule, or gene targeting molecule of the present invention. The requirements for expressing such molecules are similar to those for expressing a recombinant polypeptide as described *supra*.

The present invention further extends to the production and use of starches and proteins produced using the novel genes described herein. Modified starches produced by plants which have been selected using marker-assisted selection, or alternatively, produced by transgenic plants carrying the introduced starch synthase genes, are particularly suitable for use in food products, such as, for example, flour and flour-based products, in particular those products selected from the group consisting of: flour-based sauce; leavened bread; unleavened bread; pasta, noodle; cereal; snack food; cake; and pastry. Modified proteins are also suitable for use in non-

food products, such as, for example, those non-food products selected from the group consisting of: films; coatings; adhesives; building materials; and packaging materials.

Additionally, starch hydrolysates or undegraded starches are both useful in industry and, as a consequence, the present invention is useful in applications relating to the use of both starch hydrolysates and undegraded starches. By "starch hydrolysates" is meant the glucose and glucan components that are obtainable by the enzymatic or chemical degradation of starch in chemical modifications and processes, such as fermentation.

Starch produced by plants expressing the sense, antisense, co-suppression, gene-targetting or ribozyme molecules of the present invention may exhibit modified viscosities and/or gelling properties of its glues when compared to starch derived from wild-type plants. Native starches produced by the performance of the inventive method are useful as an additive in the following: (i) foodstuffs, for the purpose of increasing the viscosity or gelling properties of food; (ii) in non-foodstuffs, such as an adjuvant or additive in the paper and cardboard industries, for retention or as a size filler, or as a solidifying substance or for dehydration, or film coating, amongst others; (iii) in the adhesive industry as pure starch glue, as an additive to synthetic resins and polymer dispersions, or as an extenders for synthetic adhesives; (iv) in the textile and textile care industries to strengthen woven products and reduce burring or to thicken dye pastes; (v) in the building industry, such as a binding agent in the production of gypsum plaster boards, or for the deceleration of the sizing process; (vi) in ground stabilization or for the temporary protection of ground particles against water in artificial earth shifting; (vii) as a wetting agent in plant protectants and fertilizers; (viii) as a binding agent in drugs, pharmaceuticals and medicated foodstuff such as vitamins, etc; (ix) as an additive in coal and briquettes; (xi) as a flocculent in the processing of coal ore and slurries; (xii) as a binding agent in casting processes to increase flow resistance and improve binding strength; and (xiii) to improve the technical and optical quality of rubber and plastic products. Additional applications are not excluded.

A further aspect of the present invention provides an isolated promoter that is operable

in the endosperm of a monocotyledonous plant cell, tissue or organ, and preferably in the endosperm of a monocotyledonous plant cell, tissue or organ. According to this embodiment, it is preferred that the promoter is derived from a starch synthase gene of the present invention, such as a promoter that is linked *in vivo* to any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38, or a complementary nucleotide sequence thereto.

In a particularly preferred embodiment, the promoter comprises a nucleotide sequence derivable from the 5'-upstream region of SEQ ID NO: 11 or SEQ ID NO: 37 or SEQ ID NO: 38, or a complementary nucleotide sequence thereto, an more preferably comprises nucleotides 1 to about 287 of SEQ ID NO: 11, or nucleotides 1 to about 1416 of SEQ ID NO: 37, or nucleotides 1 to about 973 of SEQ ID NO: 38, or a complementary nucleotide sequence thereto. The present invention clearly extends to promoter sequences that comprise further nucleotide sequences in the region upstream of the stated nucleotide sequence that are linked *in vivo* to said nucleotide sequence in the wheat genome.

In a related embodiment, the promoter sequence of the present invention will further comprise an exon sequence derived from a starch synthase gene, such as, for example, an intron I sequence described herein, or a complementary nucleotide sequence thereto. Those skilled in the art will be aware that the inclusion of such nucleotide sequences may increase the expression of a heterologous structural gene, the expression of which is controlled thereby. Preferred intron I sequences include, for example, nucleotide sequences in the region of about position 1744 to about 1847 of SEQ ID NO: 37, and/or about position 1100 to about position 2056 of SEQ ID NO: 38. Additional sequences comprising intron/exon junction boundary sequences which are readily determined by those skilled in the art are not excluded.

The present invention further extends to the expression of any structural gene operably under the control of the starch synthase promoter sequence exemplified herein or a functional homologue, analogue or derivative of said promoter sequence.

As with other embodiments described herein for expression in cells, a genetic construct may be employed to effect said expression and the present invention clearly extends to said genetic constructs.

The polypeptide encoded by the structural gene component may be a reporter molecule which is encoded by a gene such as the bacterial β-glucuronidase gene or chloramphenicol acetyltransferase gene or alternatively, the firefly luciferase gene. Alternatively, wherein it is desirable to alter carbon partitioning within the endosperm, the polypeptide may be an enzyme of the starch sucrose biosynthetic pathways. Preferably, the promoter sequence is used to regulate the expression of one or more of the starch synthase genes of the present invention or a sense, antisense, ribozyme, co-suppression or gene-targetting molecule comprising or derived from same.

Recombinant DNA molecules carrying the aforesaid nucleic acid molecule of the present invention or a sense, antisense, ribozyme, gene-targetting or co-suppression molecule and/or genetic construct comprising same, may be introduced into plant tissue, thereby producing a "transgenic plant", by various techniques known to those skilled in the art. The technique used for a given plant species or specific type of plant tissue depends on the known successful techniques. Means for introducing recombinant DNA into plant tissue include, but are not limited to, transformation (Paszkowski *et al.*, 1984), electroporation (Fromm *et al.*, 1985), or microinjection of the DNA (Crossway *et al.*, 1986), or T-DNA-mediated transfer from *Agrobacterium* to the plant tissue. Representative T-DNA vector systems are described in the following references: An *et al.*(1985); Herrera-Estrella *et al.* (1983a, b); Herrera-Estrella *et al.* (1985). Once introduced into the plant tissue, the expression of the introduced gene may be assayed in a transient expression system, or it may be determined after selection for stable integration within the plant genome. Techniques are known for the *in vitro* culture of plant tissue, and in a number of cases, for regeneration into whole plants. Procedures for transferring the introduced gene from the originally transformed plant into commercially useful cultivars are known to those skilled in the art.

In general, plants are regenerated from transformed plant cells or tissues or organs on

hormone-containing media and the regenerated plants may take a variety of forms, such as chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., a transformed root stock grafted to an untransformed scion in citrus species). Transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plants may be selfed to give homozygous second generation (or T2) transformed plants, and the T2 plants further propagated through classical breeding techniques.

Accordingly, a still further aspect of the present invention contemplates a transgenic plant comprising an introduced sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37, or 38, or a complementary nucleotide sequence thereto or a genetic construct comprising same. The present invention further extends to those plant parts, propagules and progeny of said transgenic plant or derived therefrom, the only requirement being that said propagules and progeny also carry the introduced nucleic acid molecule(s).

The present invention is further described by reference to the following non-limiting examples.

EXAMPLE 1

Plant material

Genetic stocks of hexaploid bread wheat *Triticum aestivum* cv. Chinese Spring with various chromosome additions and deletions were kindly supplied by Dr E. Lagudah (CSIRO Plant Industry, Canberra) and derived from stocks described in Sears and Miller (1985). The hexaploid (*Triticum aestivum*) wheats cv Gabo and cv Wyuna were grown in controlled growth cabinet conditions (18°C day and 13 C night, with a photoperiod of 16 h). Wheat leaves and florets prior to anthesis, and endosperm were collected over the grain filling period, immediately frozen in liquid nitrogen and stored

at -80°C until required.

EXAMPLE 2

Gel Electrophoresis, Antibodies and Immunoblotting

Monoclonal antibodies against the Sgp-1 proteins, and their use in the immunoblotting of SDS-PAGE gels have been described previously (Rahman *et al.*, 1995).

EXAMPLE 3

Preparation of total RNA from wheat

Total RNA was isolated from the leaf, floret and endosperm tissues of wheat essentially as described by Higgins *et al.* (1976) or Rahman *et al.* (1998). RNA was quantified by UV absorption and by separation in 1.4% (w/v) agarose-formaldehyde gels which were then visualised under UV light after staining with ethidium bromide.

EXAMPLE 4

Construction and screening of cDNA libraries

A first cDNA library, an expression cDNA library of wheat endosperm, was constructed from mRNA isolated from wheat cv Chinese Spring. RNA from 5, 7, 9, 11 and 13 days after anthesis was pooled and random primers were used for the first strand of cDNA synthesis. Monoclonal antibodies against 100 -105 kDa proteins in wheat starch granules (Rahman *et al.*, 1995) were used for immunoscreening of the expression cDNA library.

A second cDNA library was constructed from the endosperm mRNA of the hexaploid *Triticum aestivum* cultivar Wyuna, 8 - 12 days after anthesis, as described by Rahman *et al.* (1997). This library was screened with a 85-bp cDNA fragment, wSSIIp1, which was obtained by immunoscreening of the expression cDNA library as described above. The wSSIIp1 probe corresponded to nucleotide positions 988 to 1072 of wSSIIB (SEQ ID NO:1) at the hybridisation conditions as described earlier (Rahman *et al.*, 1998).

A third cDNA library was constructed from RNA from the endosperm of the hexaploid

Triticum aestivum cultivar Rosella as described by Rahman *et al.* (1997). This library was screened with a 347-bp cDNA fragment, wSSIIIp1 for the first screening, and a 478-bp cDNA fragment wSSIIIp3 for the second screening using the hybridisation conditions described herein.

EXAMPLE 5

Construction and screening of *Triticum tauschii* genomic library

The genomic library used in this study, prepared from *Triticum tauschii*, var strangulata, (Accession Number CPI 110799), has been described in Rahman *et al.*, (1997). Of all the accessions of *T. tauschii* surveyed, DNA marker analysis suggests that the genome of CPI 110799 is the most closely related to the D genome of hexaploid wheat (Lagudah *et al.*, 1991).

Hybridisations were carried out in 25% formamide, 6 x SSC, 0.1% SDS at 42°C for 16 hours, then filters were washed 3 times using 2 x SSC containing 0.1% SDS at 65°C for 1 hour per wash.

For the isolation of a genomic wSSII clone, the probe comprised the PCR-derived DNA fragment wSSIIp2 and positive-hybridising plaques were digested using the restriction enzyme *Bam*HI, separated on a 1% agarose gel, transferred to nitrocellulose membrane and hybridised to probe wSSIIp4 comprising nucleotides 1 to 367 of the wSSIIA cDNA clone, using the conditions described by Rahman *et al.* (1997).

For the isolation of a genomic wSSIII clone, plaques hybridising to the PCR-derived DNA fragment wSSIIIp1 from clone wSSIII.B3 (i.e. nucleotides 3620 to 3966 of SEQ ID NO:7) were selected and re-screened until plaque-purified.

EXAMPLE 6

DNA sequencing and analysis

DNA sequencing was performed using the automated ABI system with dye terminators as described by the manufacturers. DNA sequences were analysed using the GCG

suite of programs (Devereaux *et al.*, 1984).

EXAMPLE 7

DNA and RNA analysis

DNA was isolated and analysed as previously described (Maniatis *et al.*, 1982; Rahman *et al.*, 1998). Approximately 20 μg of DNA was digested with restriction enzymes *Bam*HI, *Dra*I and *Eco*RI, separated on a 1% agarose gel and transferred to reinforced nitrocellulose membranes (BioRad) and hybridised with ^{32}P-labelled DNA probe, either wSSIIIp1, corresponding to nucleotides 3620 to 3966 of the wheat SSIII gene, or alternatively, with the entire wSSII cDNA clone. DNA fragment probes were labelled with the Rapid Multiprime DNA Probe Labelling Kit (Promega).

The hybridisation and wash conditions were performed as described in Rahman *et al.* (1997). For RNA analysis, 10 μg of total RNA was separated in a 1.4% agarose-formaldehyde gel and transferred to a Hybond N+ membrane (Amersham), and hybridised with cDNA probe at 42°C as previously described by Khandjian *et al.*, (1987) or Rahman *et al.*, (1998). After washing for 30 minutes at 65°C with 2x SSC, 0.1% SDS; followed by three washes of 40 minutes at 65°C with 0.2x SSC, 1% SDS, the membranes were visualised by overnight exposure at -80°C with Kodak MR X-ray film.

EXAMPLE 8

Expression of wheat Sgp-1 polypeptides in the wheat endosperm

The development and use of monoclonal antibodies to the Sgp-1 proteins has been described previously (Rahman *et al.*, 1995). These antibodies were used by the present inventors to characterise the expression and localisation of the Sgp-1 proteins.

The proteins found in the matrix of the wheat starch granule are shown in Figure 1, lane 1. The remaining lanes show an immunoblot of proteins from the soluble phase (Figure 1; lanes 2-4) and the starch granule (Figure 1; lanes 5-7), respectively, following SDS-PAGE. In addition to cross-reactivity with the 100-105 kDa proteins, a

weak cross-reaction with a 50 kDa protein in both the granule and the soluble fractions were observed (Figure 1). The Sgp-1 polypeptides are present in the starch granule throughout endosperm development (Figure 1; lanes 5-7, also see Rahman *et al.*, 1995). However, as the endosperms matures, there is a reduction in the amount of Sgp-1 protein found in the soluble fraction. Lane 4 shows that by 25 days after anthesis, the level of these proteins in the soluble fraction is substantially reduced. This observation is consistent with previous results from Rahman *et al.*, (1995), who suggested that the Sgp-1 proteins were exclusively granule bound based on studies of granules from endosperm in mid-late stages endosperm development, however, these results suggest that the partitioning of these proteins between the granule and the soluble phase changes during development.

EXAMPLE 9

Isolation of cDNA clones encoding wheat starch synthase II (wSSII) proteins

Monoclonal antibodies against Sgp-1 polypeptides (Rahman *et al.*, 1995) were used to probe the expression library described in Example 4 (i.e. the first cDNA library). Three immunoreactive plaques were identified and sequenced. One clone, designated wSSIIp1, contained an 85-bp cDNA insert with homology to maize SSIIa (Harn *et al.*, 1998).

DNA from the wSSIIp1 clone was used as a probe in the hybridisation screening of the second cDNA library, prepared from *Triticum aestivum* cultivar Wyuna endosperm RNA as described in Example 4. Ten hybridising cDNA clones were selected and sequenced. On the basis of the DNA sequences obtained, the 10 cDNA clones can be classified into three groups. Group 1 contains 7 cDNA clones, group 2 contains 2 cDNA clones and group 3 contains 1 cDNA clone.

The longest clone from group 1 (designated wSSIIB) is 2939 bp in length (SEQ ID NO:1) and encodes a 798 -amino acid polypeptide in the region from nucleotide position 176 to nucleotide position 2569 (SEQ ID NO:2).

The longest clone from group 2 (designated wSSIIA) is 2842 bp in length (SEQ ID NO:3) and encodes a 799 -amino acid polypeptide in the region from nucleotide position 89 to nucleotide position 2485 (SEQ ID NO:4).

The cDNA from group 3 is a partial cDNA clone (designated wSSIID), which is 2107 bp in length (SEQ ID NO:5) and encodes a 597 -amino acid polypeptide in the region from nucleotide position 1 to nucleotide position 1791 (SEQ ID NO:6). The encoded polypeptide is approximately a 200 amino acid residues shorter than that of polypeptides encoded by longest clones of group 1 or 2 clones, respectively (Figure 2).

Comparison of the three cDNA clones, wSSIIB, wSSIIA and wSSIID shows that they share 95.7% to 96.6% identity at the amino acid level, with variation at 44 amino acid positions between the three sequences (Figure 3). Of the 44 amino acid changes between these sequences, 31 changes occur in the N-terminal region (residues 1 to 300), 10 changes occur in the central region (residues 301 to 729) and 3 changes occur in the C-terminal region (residues 730 to 799). The wSSIIA polypeptide (799 amino acid residues) and wSSIIB polypeptide (798 amino acid residues) sequences differ in length by a single amino acid residue, due to the deletion of Asp-69 from the wSSIIB polypeptide sequence.

A comparison of the nucleotide sequences of the wSSIA, wSSIIB and wSSIID cDNA clones with the nucleotide sequence of the wSSIIp1 cDNA obtained by immunoscreening confirms that the wSSIIp1 sequence is found in each cDNA (Figure 3). The peptide encoded by the wSSIIp1 cDNA clone corresponds to amino acid residues in the region from residue 272 to residue 298 of the wSSIIA polypeptide, and to amino acid residues in the region from residue 271 to residue 297 of the wSSIIB polypeptide (see Figure 3). Thus, the peptide epitope encoded by wSSIIp1 that reacts with the anti-Sgp-1 monoclonal antibodies can therefore be localised to this region of the wSSIIA and wSSIIB polypeptides and to the corresponding region of the wSSIID polypeptide.

Notwithstanding that a region having about 63% amino acid sequence identity to the peptide epitope encoded by clone wSSIIp1 is found in the maize SSIIa polypeptide (Figure 3), the degree of amino acid conservation between maize and wheat sequences in this region of the polypeptide is insufficient for immunological cross-reactivity to occur between these species using the monoclonal antibodies to the wheat Sgp-1 proteins described by Rahman *et al.* (1995). Additionally, this peptide epitope is not found in granule-bound starch synthases, SSI, or SSIII (data not shown).

The wSSIIB cDNA (SEQ ID NO:1) encodes an amino acid sequence comprising the peptide motif AAGKKDAGID (SEQ ID NO: 18) between residues 60 and 69 of SEQ ID NO:2 (Figure 3) which, with the exception of the second residue, is identical to the N-terminal of the 100 kDa ($A^T/_L$GKKDAGID: SEQ ID NOS:19 and 20) protein (Sgp-B1) from the wheat starch granule (note that the sequence given in Rahman *et al.*, 1995 ($A^T/_L$GKKDAL: SEQ ID NOS: 21 and 22) has been revised following further amino acid sequence analysis).

The wSSIIA cDNA clone (SEQ ID NO:3) encodes an amino acid sequence comprising the peptide motif AAGKKDARVDDDAA (SEQ ID NO: 23) at residues 60 to 73 of SEQ ID NO:4, which is about 66% identical to the N-terminal amino acid sequence (i.e. ALGKKDAGIVDGA: SEQ ID NO: 24) of the 104 kDa and 105 kDa starch granule proteins, Sgp-D1 and Sgp-A1 respectively, as determined by sequence analysis of isolated protein (Rahman *et al.*, 1995).

Furthermore, Takaoka *et al.* (1997) reported the amino acid sequences of 3 polypeptides obtained from sequencing starch granule proteins derived from the Sgp-1 proteins. Peptide 3 described by Takaoka *et al.* (1997) corresponds to amino acid residues 378 to 387 of the amino acid sequence of the wSSIIA cDNA (SEQ ID NO:4; Figure 3). Peptides 1 and 2 described by Takaoka *et al.* (1997) could not be detected in the amino acid sequences of the wSSII cDNA clones of the present invention, however peptide 1 of Takaoka *et al.* (1997) can be found in the amino acid sequences of SSI from maize, rice, wheat and potato (data not shown).

Denyer *et al.* (1995) demonstrated that the Sgp-1 proteins possess starch synthase activity and, as a consequence, the wSSIIB, wSSIA and wSSIID cDNA clones encode starch synthase enzymes that are differentially expressed in a developmentally-regulated manner in both the soluble and granule-bound fractions of the endosperm (Figure 1). Based on the nomenclature suggested by Harn *et al.* (1998), it is appropriate to describe the Sgp-1 proteins as "starch synthases" rather than "granule-bound starch synthases".

EXAMPLE 10

Analysis of wheat starch synthase II mRNA expression

The mRNA for wheat starch synthase II could be detected in leaves, pre-anthesis florets and endosperm of wheat when total RNAs isolated from these tissue were probed with a PCR probe, wSSIIp2, corresponding to nucleotide positions 1435 to 1835 bp of wSSIIB-cDNA (SEQ ID NO:1; Figure 4). Unlike wSSI, which could not be detected in wheat leaves derived from plants grown under the same conditions, wSSII genes are highly-expressed in the leaves (Figure 4, lane 1), and expressed at an intermediate level in pre-anthesis florets (Figure 4, lane 2), and at much lower levels in developing wheat endosperm cells (Figure 4, lanes 3-11). In contrast, the maize SSIIa is expressed predominantly in the endosperm, whilst the maize SSIIb is detected mainly in the leaf, albeit at low levels (Harn *et al.*, 1998).

The wSSII mRNA was detectable in the endosperm 6 days after anthesis and mRNA levels increase between 8 and 18 days post-anthesis, after which time levels of mRNA decline.

Southern blotting experiments in wheat demonstrated that the wSSIIp2 probe used detected only a single copy of the SSII gene in each genome (data not shown). Thus, it is unlikely that this probe cross-hybridised with mRNAs encoded by genes other than wSSII.

EXAMPLE 11

Chromosomal localization of the wheat wSSII genes.

I. Amplification of specific cDNA regions of wheat starch synthase II using PCR

Two PCR products, wSSIIp2 and wSSIIp3 were amplified from the cDNA clone wSSIIB and used for the northern hybridisation and Southern hybridisation, respectively.

The primers sslIa (5' TGTTGAGGTTCCATGGCACGTTC 3': SEQ ID NO: 25) and sslIb (5' AGTCGTTCTGCCGTATGATGTCG 3': SEQ ID NO: 26) were used to amplify the cDNA fragment wSSIIp2 (i.e. nucleotide positions 1435 to 1835 of SEQ ID NO:1).

The primers sslIc (5' CCAAGTACCAGTGGTGAACGC 3': SEQ ID NO: 27) and sslId (5' CGGTGGGATCCAACGGCCC 3': SEQ ID NO: 28) were used to amplify the cDNA fragment wSSIIp3 (i.e. nucleotide positions 2556 to 2921 of SEQ ID NO:1).

The amplification reactions were performed using a FTS-1 thermal sequencer (Corbett, Australia) for 1 cycle of 95°C for 2 minutes; 35 cycles of 95°C for 30 seconds, 60°C for 1 minutes, 72°C for 2 minutes and 1 cycle of 25°C for 1 minute.

II. PCR and nucleotide sequence analysis of 3' sequences of wheat SSII genes

Genomic DNA was extracted from wild-type Chinese Spring wheat, and from three nullisomic-tetrasomic lines of chromosome 7 of Chinese Spring wheat, and from *Triticum tauschii* (var strangulata, accession number CPI 100799), and used as a template for the amplification and nucleotide sequence analysis of wheat SSII genes.

RFLP analysis of *Bam*HI and *Eco*RI restricted DNA from each wheat or *T. Tauschii* line was carried out using the wSSIIp3 fragment as a probe. Three hybridising bands were obtained which could be assigned to chromosomes 7A, 7B and 7D, respectively (data not shown). This analysis indicates that there is a single copy of the wSSII gene in each genome in hexaploid wheat, consistent with the findings of Yamamori and Endo (1996) who located the SGP-A1, B1 and D1 proteins to the short arm of chromosome 7.

PCR analysis was used to assign each of the cDNA clones to the individual wheat genomes. A single 365 bp PCR fragment was obtained from nullisomic-tetrasomic genomic DNA of Chinese Spring when primers ssIIc and ssIId were used for the PCR amplification (Figure 5, right panel). This PCR product is obtained only from lines bearing the B genome. The fragment was cloned and sequenced and shown to be identical to a 365 bp region of the wSSIIB cDNA. An identical fragment is obtained by PCR amplification of the wSSIIB cDNA clone, but not by amplification of the wSSIIA or wSSIID clones, supporting the conclusion that the wSSIIB cDNA is the product of a gene located on chromosome 7 of the B genome of hexaploid wheat.

Two PCR products were also amplified from nullisomic-tetrasomic genomic DNA of Chinese Spring using the primers ssIIc and ssIIe (Figure 5, left panel). One PCR fragment, approximately 350 bp is only amplified when the A genome is present, and a second 322 bp product is only amplified when the D-genome is present. The 350 and 322 bp PCR products were also cloned and sequenced and shown to be identical to the wSSIIA and wSSIID cDNAs, respectively, supporting the conclusion that the wSSIIA and wSSIID cDNAs are the products of genes located on chromosomes 7A and 7D, respectively.

EXAMPLE 12

Isolation of genomic wSSII clones

Screening of a genomic library from the D-genome donor of wheat, *T. tauschii*, was performed as described in Example 5, using the PCR-derived DNA fragment wSSIIp2 as a hybridisation probe. A positive-hybridising clone, designated wSSII-8, and comprising a putative *T. tauschii* homologue of the wSSII gene, was isolated.

Positive-hybridising plaques were digested using the restriction enzyme *Bam*HI, separated on a 1% agarose gel, transferred to nitrocellulose membrane and hybridised to probe wSSIIp4 comprising nucleotides 1 to 367 of the wSSIIA cDNA clone, using the conditions described by Rahman *et al.* (1997). Clone wSSII-8 also hybridises strongly to the wSSIIp4 probe, confirming its identity as a genomic wSSII gene.

The complete nucleotide sequence of the wSSII gene was determined and is presented herein as SEQ ID NO: 37. The structural features of this gene are present in Table 3. A schematic representation of the intron/exon organisation of this gene is also presented in Figure 6.

TABLE 3

Structural features of the wheat starch synthase II genomic gene

Nucleotide Position in SEQ ID NO: 37	Feature	Length (bases)
1- 1416	5'-untranscribed region and promoter sequence	1416
1417 - 1743	exon 1	327
1480-1482	translation start codon (ATG)	3
1744 - 1847	intron 1	104
1848 - 2553	exon 2	706
2554 - 2641	intron 2	88
2642 - 2706	exon 3	65
2707 - 3606	intron 3	900
3607 - 3684	exon 4	78
3685 - 3773	intron 4	89
3774 - 3884	exon 5	111
3885 - 3981	intron 5	97
3982 - 4026	exon 6	45
4027 - 4406	intron 6	380
4407 - 4580	exon 7	174
4581 - 7296	intron 7	2716
7297 - 8547	exon 8	1251
8251 - 8253	translation stop codon (TGA)	3
8548 -9024	3'-untranscribed region	477

EXAMPLE 13

Cloning of specific cDNA regions of wheat starch synthase III using RT-PCR

PCR primers were used to amplify sequences of starch synthase III from wheat endosperm cDNA. The design of PCR primers was based on the sequences of starch synthase III from potato and the *du1* starch synthase III gene of maize.

First-strand cDNAs were synthesised from 1 μg of total RNA (derived from endosperm of the cultivar Rosella, 12 days after anthesis) as described by Maniatis *et al.* (1982), and then used as templates to amplify two specific cDNA regions, wSSIIIp1 and wSSIIIp2, of wheat starch synthase III by PCR.

The primers used to obtain the cDNA clone wSSIIIp1 were as follows:
Primer wSS3pa (5' GGAGGTCTTGGTGATGTTGT 3': SEQ ID NO: 29); and
Primer wSS3pb (5' CTTGACCAATCATGGCAATG 3': SEQ ID NO: 30).

The primers used to obtain the cDNA clone wSSIIIp2 were as follows:
Primer wSS3pc (5' CATTGCCATGATTGGTCAAG 3': SEQ ID NO: 31); and
Primer wSS3pd (5' ACCACCTGTCCGTTCCGTTGC 3': SEQ ID NO: 32).

The amplified clones wSSIIIp1 and wSSIIIp2 were used as probes to screen the third cDNA library and *T. tauschii* genomic DNA library as described in Example 4.

A further probe designated wSSIIIp3 was used for screening the third cDNA library, as described in Example 4. Probe wSSIIIp3 was amplified by PCR from a cDNA clone produced from the first screening using the following amplification primers:
Primer wSS3pe (5' GCACGGTCTATGAGAACAATGGC 3': SEQ ID NO: 33); and
Primer wSS3pf (5' TCTGCATACCACCAATCGCCG 3': SEQ ID NO: 34).

The amplification reactions were performed using a FTS-1 or FTS4000 thermal sequencer (Corbett, Australia) for 1 cycle of 95°C for 2 minutes; 35 cycles of 95°C for 30 seconds, 60°C for 1 minutes, 72°C for 2 minutes and 1 cycle of 25°C for 1 minute.

Amplified sequences of the expected length were obtained, cloned and sequenced, and shown to contain DNA sequences highly homologous to the maize and potato SSIII genes. PCR fragments were subsequently used to probe a wheat cDNA library by DNA hybridisation and 8 positive clones were obtained, including one 3 kb cDNA. A region from the 5' end of this cDNA was amplified by PCR and used a probe for a second round of screening the cDNA library, obtaining 8 cDNA clones. Of these, one cDNA was demonstrated to be full length (wSSIII.B3, 5.36 kb insert). The sequence of the 5,346 bp wSSIII.B3 cDNA clone is given in SEQ ID NO:7.

Sequencing of the 8 cDNA clones obtained from the second round screening of the wheat cDNA library revealed that there were at least 2 classes of cDNA encoding SSIII present, possibly being encoded by homeologous genes on different wheat genomes. The sequence of a representative of this second class of cDNA clones, wSSIII.B1, is shown in SEQ ID NO:9. The 3261 bp clone wSSIII.B1 is not full length, however it is similar to nucleotides 1739 to 5346 of the homeologous clone wSSIII.B3 (SEQ ID NO: 7). Clone wSSIII.B1 has an open reading frame between nucleotide positions 1 and 3177.

An open reading frame is found in the cDNA clone wSSIII.B3 (SEQ ID NO:7), in the region between position 29, commencing the ATG start codon, and nucleotide position 4912. The amino acid sequence deduced from this open reading frame is shown in SEQ ID NO:8.

An alignment of the deduced amino acid sequences of SSIII from maize, potato and wheat is shown in Figure 7. There is about 56.6% identity between the maize SSIII and wheat wSSIII.B3 sequence at the amino acid level.

The C-terminal domain of starch synthases comprise the catalytic domain, and a characteristic amino acid sequence motif KVGGLGDVVTSLSRAVQDLGHNVEV (SEQ ID NO: 35) in maize, or alternatively KVGGLGDVVTSLSRAIQDLGHTVEV (SEQ ID

NO: 36) in wheat, marking the first conserved region in the C-terminal domain. This amino acid sequence is present at amino acid residues 1194 to 1218 of SEQ ID NO: 8.

The amino acid identity between maize dull1 and wSSIII.B3 in the N-terminal region (i.e. amino acids 1 to 600 in Figure 7) is only 32.2%; whilst the amino acid identity in the central region (i.e. amino acids 601 to 1248 in Figure 7) is 68.4%; and in the C-terminal region (i.e. amino acids 1249 to 1631 in Figure 7) is 84.6%. Accordingly, the SSIII starch synthases are much more highly conserved between maize and wheat in the region comprising the catalytic domain of the proteins.

EXAMPLE 14

Analysis of wheat starch synthase III mRNA expression

Figure 8 shows the expression of wSSIII mRNA during endosperm development in two wheat varieties grown under defined environmental conditions. The expression of the gene is seen very early in endosperm development in both cultivars, 4 days after anthesis (Figure 8, panels a and b). Expression in the leaf of the variety Gabo is very weak (Figure 8, panel c, Lane L) whereas strong expression is seen in pre-anthesis florets (Figure 8, panel c, Lane P).

EXAMPLE 15

Amino acid sequence comparisons between wheat SSII and SSIII polypeptides

Amino acid sequence comparisons between wheat BSSS, SSI, SSII and SSIII polypeptides reveals eight highly-conserved domains (Figure 9). The amino acid sequences of these domains are represented in the wheat SSIII amino acid sequence by the following sequence motifs:

(a) Region 1: KVGGLGDVVTS;

(b) Region 2: GHTVEVILPKY;

(c) Region 3: HDWSSAPVAWLYKEHY;

(d) Region 4: GILNGIDPDIWDPYTD;

(e) Region 5: DVPIVGIITRLTAQKG;
(f) Region 5a: NGQVVLLGSA;
(g) Region 6: AGSDFIIVPSIFEPCGLTQLVAMRYGS; and
(h) Region 7: TGGLVDTV.

These conserved amino acid sequences are summarised in Table 4. As shown in Table 4 below, there is at least about 25% amino acid sequence identity, preferably at least about 30% amino acid sequence identity, more preferably at least about 35% amino acid sequence identity, more preferably at least about 40% amino acid sequence identity, more preferably at least about 45% amino acid sequence identity, more preferably at least about 50% amino acid sequence identity, more preferably at least about 55% amino acid sequence identity, more preferably at least about 60% amino acid sequence identity, more preferably at least about 65% amino acid sequence identity, more preferably at least about 70% amino acid sequence identity, more preferably at least about 75% amino acid sequence identity, more preferably at least about 80% amino acid sequence identity, more preferably at least about 85% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity and even more preferably at least about 95% amino acid sequence identity between the amino acid sequences of plant starch synthase enzymes, in particular wheat starch synthases.

From the data presented in Table 4, the most conserved regions of the wheat SSII and SSIII polypeptides are a region of 6 or 7 identical amino acids in Region 1 and a region of 8 or 9 identical amino acids in Region 6. The lowest regions of identity are found in regions 3 and 5a.

For each of the amino acid sequences presented in the first column of Table 4, which are specific for wSSIII polypeptides, corresponding signature motifs which are specific for wSSII-A, wSSII-B, and wSSII-D polypeptides can be derived from the alignment, as follows:

Region 1: KTGGLGDVAGA;

Region 2: GHRVMVVVPRY;
Region 3: NDWHTALLPVYLKAYY;
Region 4: GIVNGIDNMEWNPEVD;
Region 5: DVPLLGFIGRLDGQKG;
Region 5a: DVQLVMLGTG;
Region 6: AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
Region 7: VGG(V/L)RDTV.

Comparison of the amino acid sequences of all available starch synthases with the deduced amino acid sequences of the three wSSII cDNA clones of the present invention (i.e. wSSIIB, wSSIIA and wSSIID) was conducted using PILEUP analysis (Devereaux *et al.*, 1984) and data are presented herein as a dendrogram (Figure 10). The sequence of the glycogen synthase of *E. coli* was also included. Based upon their amino acid similarities, four classes of plant starch synthases can be defined: GBSS, SSI, SSII and SSIII.

Table 5 shows that levels of identity at the amino acid level between the wSSII sequences, as determined using the BESTFIT programme in GCG (Devereaux *et al.*, 1984), and other class II starch synthases range from 70% identity with potato SSII to 85% identity with maize SSIIa. Both wSSIIB and wSSIID showed significantly higher homology to maize SSIIa than wSSIIA. Based upon sequence identities and the function of the Sgp-1 proteins in wheat, the wSSIIB, wSSIIA and wSSID cDNA clones are members of the starch synthase II (SSII) group and are more similar in sequence to maize SSIIa than maize SSIIb.

TABLE 4

Identities between conserved motifs of plant starch synthases

Sequence in wSSIII polypeptide	Number of conserved residues between wheat starch synthases	Number of conserved residues between wheat SSII and SSIII polypeptides
Region 1: KVGGLGDVVTS	6/11 residues	6/11 residues
Region 2: GHTVEVILPKY	6/11 residues	6/11 residues
Region 3: HDWSSAPVAWLYKEHY	4/16 residues	5/16 residues
Region 4: GILNGIDPDIWDPYTD	7/16 residues	8/16 residues
Region 5: DVPIVGIITRLTAQKG	8/16 residues	10/16 residues
Region 5a: NGQVVLLGSA	4/10 residues	4/10 residues
Region 6: AGSDFIIVPSIFEPCGLT QLVAMRYGS	15/27 residues	17/27 residues
Region 7: TGGLVDTV	5/9 residues	5/9 residues

TABLE 5

	wSSII-A	wSSII-B	wSSII-D
wSSI-A	100%		
wSSII-B	95.9%	100%	
wSSII-D	96.3%	96.7%	100%
maize SSIIa	76.1%	85.2%	84.7%
maize SSIIb	76.3%	76.7%	75.9%
pea SSII	72.0%	72.2%	71.8%
potato SSII	70.9%	71.1%	70.3%

Figure 11 shows a schematic representation of an alignment of plant starch synthase sequences, including wheat GBSS, wheat SSI, wheat SSII-A1, maize SSIIa, and maize dull-1 polypeptides, in which the position of the first homologous region, comprising the consensus motif KXGG, is used as the basis of the alignment. The major differences in structure between the classes of genes are found in the length of the N-terminal region between the transit peptide and the first conserved region. At one extreme, the GBSS genes have a very short N-terminal arm, whereas the *du1* starch synthase contains a very long N-terminal extension containing several distinct regions. The wSSII genes contain an N-terminal extension which is longer than either GBSS, SSI, or SSIIb, and slightly longer than the maize SSIIa gene.

EXAMPLE 16

Isolation of genomic clones for SSIII

Screening of a genomic library from the D-genome donor of wheat, *T. tauschii*, identified a number of clones which hybridised to the wSSIII PCR fragment. Positive plaques in the genomic library were selected as those hybridising with a probe that had been generated by PCR (amplifying between nucleotide positions 3620 to 3966) from the SSIII cDNA as template. The primer sequences used were as follows:

wSS3pa (5' GGAGGTCTTGGTGATGTTGT 3': SEQ ID NO: 29); and

wSS3pb (5' CTTGACCAATCATGGCAATG 3' : SEQ ID NO: 30).

Hybridisation was carried out in 25% formamide, 6 x SSC, 0.1% SDS at 42 °C for 16 hour, then washed three times with 2 x SSC containing 0.1% SDS at 65 °C, for 1 hour per wash. shows an example of a plaque lift showing positive and negative hybridisations for plaques containing the *T. tauschii* homologue of the wSSIII.B3 gene.

DNA was isolated from positive-hybridising λ clones using methods described by Maniatis *et al.* Briefly, DNA was digested using *Bam*HI or *Bgl*I and sub-cloned in to the vector pJKKmfm. DNA sequencing was performed using the automated ABI system with dye terminators as described by the manufacturers. DNA sequences were analysed using the GCG suite of programs (Devereaux *et al.*, 1984).

Nucleotide sequences of the genomic SSIII clone from *T. tauschii* are provided herein as 6 contiguous sequences designated fragments 1 to 6 (SEQ ID NOs: 11 to 16, respectively). Table 6 defines the relative positions of these fragments with respect to the SSIII cDNA and describes the positions of exons. Figure 11 shows this information schematically.

The complete nucleotide sequence of a wheat SSIII genomic gene is presented herein as SEQ ID NO: 38. The structural features of this gene are presented in Table 7. A schematic representation of the intron/exon organisation of this gene is also presented in Figure 12.

EXAMPLE 17

Discussion

Early work on the Sgp-1 starch synthase proteins (Denyer *et al.*, 1995; Rahman *et al.*, 1995) was based on the localisation of these proteins in the wheat starch granule, and no definitive conclusion concerning their presence or absence in soluble extracts of the wheat endosperm was presented.

We have now demonstrated that a monoclonal antibody against the Sgp-1 proteins cross reacts strongly with those starch synthase proteins having apparent molecular

weights of 100-105 kDa in soluble extracts, however, the appearance of these proteins in soluble extracts is dependant on the developmental stage of the endosperm material. Whilst the proteins can be detected in the soluble phase in early to mid endosperm development, little or no soluble protein remains in late endosperm development (Figure 1). This observation accounts for the failure of Rahman *et al.* (1995) to detect the protein in soluble extracts in a previous report.

Based upon the localisation of the Sgp-1 starch synthase proteins in the wheat endosperm, the following nomenclature is suggested for wheat starch synthase enzymes: wGBSS for the 60 kDa granule bound starch synthase (Wx); wSSI for the 75 kDa starch synthase I (Sgp-3); wSSII for the 100 - 105 kDa proteins (Sgp-1); and wSSIII for a soluble high molecular starch synthase.

The present invention provides cDNA and genomic clones encoding the wSSII and wSSIII polypeptides and the corresponding genomic clones. Whilst the evidence is compelling that the wSSIIA, wSSIIB and wSSIID cDNAs encode the Sgp-A1, Sgp-B1 and Sgp-D1 proteins of the wheat starch granule, molecular weights calculated from the deduced amino acid sequences of the clones are considerably lower than estimates obtained from SDS-PAGE. The molecular weight of the precursor wSSIIA protein is 87,229 Da, and the mature protein 81,164 Da, yet the estimated molecular weight in our experience is 105 kDa. The assignment of the wSSIIA cDNA to the A-genome of wheat is demonstrated in Figure 5, and the assignment of the 105 kDa protein to the A-genome in Denyer *et al.* (1995) and Yamamori and Endo (1996). Similarly, the molecular weight of the wSSIIB protein is 86,790 Da and the mature protein 80,759 Da, yet the molecular weight of the Sgp-B1 protein is estimated to be 100 kDa. No comparison can be made of the wSSIID sequences as a full length cDNA clone was not obtained. The wSSIIA and wSSIIB amino acid sequences differ by just a single amino acid residue, yet there is an apparent difference of 5 kDa in molecular weight when estimated by SDS-PAGE. Several possibilities can be advanced to account for this apparent discrepancy in molecular weights. Firstly, the wSSII proteins may not migrate in SDS-PAGE in accordance with their molecular weight because they

retain some conformation under the denaturing conditions used. Secondly, the proteins may be glycosylated. It is also possible that the proteins may be non-covalently linked to starch through a high affinity starch binding site which survives denaturation and SDS-PAGE. Differences between the apparent molecular weights and those calculated from the deduced amino acid sequences will have to be defined in establishing the relationship between the wSSII proteins and proteins encoded by the analogous SSII genes of other species.

The catalytic domain of the starch synthases is found at the C-terminal end of the protein (Gao *et al.*, 1998; Harn *et al.*, 1998). Harn *et al.* (1998) identified 7 conserved regions among SSIIa, SSIIb, SSI and GBSS sequences. We have identified an additional conserved region (designated region 5a in Table 4 and Figure 10) comprising the amino acid sequence motif DVQLVMLGTG, by a comparison of the wSSII and wSSIII sequences of the present invention with differing isoforms of other plant starch synthases (GBSS, SS1, SSII and SSIII). The conservation of eight peptide regions among the 4 classes of starch synthases is striking, in terms of their sequence homologies and their alignment.

Analysis of the wheat SSII genes shows that there is a motif, PVNGENK, which is repeated. The area surrounding the repeated PVNGENK motif is not homologous to maize SSIIa and the insertion of this region is responsible for the difference in length between the wheat SSII and maize SSIIa genes. In pea and potato SSII polypeptides, a PPP motif (Figure 3; residues 251-253 and 287-289 respectively) has been suggested to mark the end of the N-terminal region and to facilitate the flexibility of an "N-terminal arm". This motif is not found in either the maize or wheat SSII sequences.

The generation of a wheat line combining null alleles at each of the three wSSII loci, wSSIIA, wSSIIB and wSSIID, has been reported recently by Yamamori (1998). In this triple null line, the large starch granules were reported to be mostly deformed and a novel starch with high blue value was observed when stained with iodine, indicating that wSSII is a key enzyme for the synthesis of starch in wheat. Further analysis of the

starch derived from this triple null mutant is in progress.

Mutations in starch synthases are known in three other species. In pea, mutation in SSII gives rise to starch with altered granule morphology and an amylopectin which yields an oligosaccharide distribution with reduced chain length on debranching, compared to the wild type (Craig *et al.*, 1998). A similar mutation in a gene designated SSII is known in *Chlamydomonas* (the *sta*-3 mutation) and similar effects on granule morphology and amylopectin structure are observed (Fontaine *et al.*, 1993). In maize, two mutations affecting starch synthases are known. First, the *dull1* mutation has been shown to be caused by a lesion within the *du*1 SSIII-type starch synthase gene (Gao *et al.*, 1998). A second mutation, the *sugary*-2 mutation yields a starch with reduced amylopectin chain lengths on debranching (this mutation co-segregates with the SSIIa locus (Harn *et al.*, 1998) although direct evidence that the *sugary*-2 mutation is caused by a lesion in the SSIIa gene is lacking). In the SSII mutants of each of these species, amylose biosynthesis capacity is retained, suggesting different roles in amylose and amylopectin synthesis for the GBSS and SSII genes. Given the conservation in overall organisation of the GBSS and SSII genes (see Figures 12 and 13), when an alignment is made based on the KTGGL motif of the first conserved region, this focuses attention on the role(s) of the N-terminal region in defining substrate specificity and the localisation of the proteins as the N-terminal region is the major area of divergence between the 4 classes of starch synthases. However, it is premature to exclude the influence of more subtle mutations in central and C-terminal regions of the gene.

The cloning of the wSSII and wSSIII cDNAs and genomic clones described herein provides useful tools for the further study of the roles of the starch synthases in wheat. Firstly, they provide a source of markers which can be used to recover and combine null or divergent alleles. Secondly, genetic manipulation of wheat by gene suppression or over-expression can be carried out, and the genes may be used for over expression in other species. The promoter regions of these genes are also useful in regulating the expression of starch synthase genes and other heterologous genes in the developing wheat endosperm and in pre-anthesis florets of wheat.

TABLE 6

Summary of the Wheat Starch Synthase III Genomic Sequence

Fragment in genomic DNA clone	Length (bp)	Features in SEQ ID NOS:11 to 16	Corresponding region in cDNA sequence
Fragment 1 (SEQ ID NO: 11)	728	Translation start codon (nucleotides 287 to 289); Exon 1.1 (nucleotides 260 to 385).	Exon 1.1: nucleotides 1 to 126
Fragment 2 (SEQ ID NO: 12)	2446	Exon 2.1 (nucleotides 1 to 1938); Exon 2.2 (nucleotides 2197 to 2418).	Exon 2.1: nucleotides 1008 to 2948; Exon 2.2: nucleotides 2949 to 3171
Fragment 3 (SEQ ID NO: 13)	1032	Exon 3.1 (nucleotides 310 to 580)	Exon 3.1: nucleotides 3172 to 3440
Fragment 4 (SEQ ID NO: 14)	892	Exon 4.1 (nucleotides 678 to 853)	Exon 4.1: nucleotides 3441 to 3616
Fragment 5 (SEQ ID NO: 15)	871	Partial Exon 5.1 (nucleotides 1 to 29) Exon 5.2 (nucleotides 293 to 463) Exon 5.3 (nucleotides 589 to 695)	Exon 5.1: nucleotides 3908 to 3937 (partial) Exon 5.2: nucleotides 3938 to 4108 Exon 5.3: nucleotides 4109 to 4215
Fragment 6 (SEQ ID NO: 16)	1583	Exon 6.1 (nucleotides 471 to 653); Exon 6.2 (nucleotides 770 to 902); Exon 6.3 (nucleotides 999 to 1110); Exon 6.4 (nucleotides 1201 to 1328); Partial Exon 6.5 (nucleotides 1408 to 1583); Translation stop codon (nucleotides 1536 to 1538)	Exon 6.1: nucleotides 4238 to 4420 Exon 6.2: nucleotides 4421 to 4552 Exon 6.3: nucleotides 4553 to 4664 Exon 6.4: nucleotides 4665 to 4793 Exon 6.5: nucleotides 4794 to 4966 (partial)

TABLE 7

Structural features of the wheat starch synthase III genomic gene

Nucleotide Position in SEQ ID NO: 38	Feature	Length (bases)
1- 973	5'-untranscribed region and promoter sequence	973
974 - 1099	exon 1	126
1001-1003	translation start codon (ATG)	3
1100 - 2056	intron 1	957
2057 - 2120	exon 2	64
2121 - 2588	intron 2	468
2589 - 5291	exon 3	2703
5292 - 5549	intron 3	258
5550 - 5767	exon 4	218
5768 - 6103	intron 4	336
6104 - 6374	exon 5	271
6375 - 7148	intron 5	774
7149 - 7324	exon 6	176
7325 - 7438	intron 6	114
7439 - 7546	exon 7	108
7547 - 7792	intron 7	246
7793 - 7902	exon 8	110
7903 - 8797	intron 8	895
8798 - 8900	exon 9	103
8901 - 9164	intron 9	264
9165 - 9335	exon 10	171
9336 - 9460	intron 10	125
9461 - 9589	exon 11	129
9590 - 9677	intron 11	88

9678 - 9860	exon 12	183
9861 - 9977	intron 12	117
9978 - 10109	exon 13	132
10110 - 10205	intron 13	96
10206 - 10317	exon 14	112
10318 - 10407	intron 14	90
10408 - 10536	exon 15	129
10537 - 10618	intron 15	82
10619 - 11146	exon 16	128
10744 - 10746	translation stop codon (TGA)	3
11147 - 11611	3'-untranscribed region	465

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CLAIMS:

1. An isolated nucleic acid molecule which comprises a sequence of nucleotides selected from the group consisting of:

(i) the nucleotide sequence set forth in SEQ ID NO: 1 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(ii) the nucleotide sequence set forth in SEQ ID NO: 3 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(iii) the nucleotide sequence set forth in SEQ ID NO: 5 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(iv) the nucleotide sequence set forth in SEQ ID NO: 7 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(v) the nucleotide sequence set forth in SEQ ID NO: 9 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(vi) the nucleotide sequence set forth in SEQ ID NO: 11 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(vii) the nucleotide sequence set forth in SEQ ID NO: 12 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(viii) the nucleotide sequence set forth in SEQ ID NO: 13 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(ix) the nucleotide sequence set forth in SEQ ID NO: 14 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(x) the nucleotide sequence set forth in SEQ ID NO: 15 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(xi) the nucleotide sequence set forth in SEQ ID NO: 16 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(xii) the nucleotide sequence set forth in SEQ ID NO: 37 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(xiii) the nucleotide sequence set forth in SEQ ID NO: 38 or the protein-encoding

region thereof or a degenerate nucleotide sequence thereto;

(xiv) the nucleotide sequence set forth in SEQ ID NO: 11 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(xv) a nucleotide sequence which encodes a wheat starch synthase polypeptide as hereinbefore defined wherein said nucleotide sequence has at least about 85% identity overall to any one of (i) to (xiv); and

(xvi) a nucleotide sequence which is complementary to any one of (i) to (xv).

2. The isolated nucleic acid molecule according to claim 1 wherein the wheat starch synthase polypeptide further comprises one or more amino acid sequences selected from the group consisting of:

(a) KVGGLGDVVTS;

(b) GHTVEVILPKY;

(c) HDWSSAPVAWLYKEHY;

(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVVLLGSA;

(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS;

(h)TGGLVDTV;

(i) KTGGLGDVAGA;

(j) GHRVMVVVPRY;

(k) NDWHTALLPVYLKAYY;

(l) GIVNGIDNMEWNPEVD;

(m) DVPLLGFIGRLDGQKG;

(n) DVQLVMLGTG;

(o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(p)VGG(V/L)RDTV.

3. The isolated nucleic acid molecule according to claim 2 wherein the wheat starch synthase polypeptide comprises at least three of said amino acid sequences selected from the group consisting of (a) to (h).

4. The isolated nucleic acid molecule according to claim 2 wherein the wheat starch synthase polypeptide comprises at least six of said amino acid sequences selected from the group consisting of (i) to (p).

5. The isolated nucleic acid molecule according to claim 1 encoding a wheat starch synthase II polypeptide.

6. The isolated nucleic acid molecule according to claim 1 encoding a wheat starch synthase III polypeptide.

7. An isolated nucleic acid molecule encoding a starch synthase polypeptide which comprises one or more amino acid sequences selected from the group consisting of:

(a) GHTVEVILPKY;

(b) HDWSSAPVAWLYKEHY;

(c) DVPIVGIITRLTAQKG;

(d) NGQVVLLGSA;

(e)AGSDFIIVPSIFEPCGLTQLVAMRYGS;

(f)TGGLVDTV;

(g) GIVNGIDNMEWNPEVD; and

(h) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT.

8. The isolated nucleic acid molecule of claim 5 encoding a wheat starch synthase II polypeptide which comprises an amino acid sequence selected from the group consisting of:

(i) SEQ ID NO: 2;

(ii) SEQ ID NO: 4;

(iii) SEQ ID NO: 6; and

(iv) a homologue of any one of (i) to (iii) having at least about 85% identity thereto.

9. The isolated nucleic acid molecule of claim 6 encoding a wheat starch synthase III polypeptide which comprises an amino acid sequence selected from the group consisting of:

(i) SEQ ID NO: 8;

(ii) SEQ ID NO: 10; and

(iii) a homologue of (i) or (ii) having at least about 85% identity thereto.

10. A probe or primer comprising at least about 15 contiguous nucleotides in length derived from the nucleotide sequence according to claim 1.

11. The probe or primer according to claim 10 comprising a nucleotide sequence selected from the group consisting of:

(i) the nucleotide sequence set forth in SEQ ID NO: 25;

(ii) the nucleotide sequence set forth in SEQ ID NO: 26;

(iii) the nucleotide sequence set forth in SEQ ID NO: 27;

(iv) the nucleotide sequence set forth in SEQ ID NO: 28;

(v) the nucleotide sequence set forth in SEQ ID NO: 29;

(vi) the nucleotide sequence set forth in SEQ ID NO: 30;

(vii) the nucleotide sequence set forth in SEQ ID NO: 31;

(viii) the nucleotide sequence set forth in SEQ ID NO: 32;

(ix) the nucleotide sequence set forth in SEQ ID NO: 33;

(x) the nucleotide sequence set forth in SEQ ID NO: 34;

(xi) a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of:

(a) KVGGLGDVVTS;

(b) GHTVEVILPKY;

(c) HDWSSAPVAWLYKEHY;

(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVVLLGSA;

(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS;

(h)TGGLVDTV;

(i) KTGGLGDVAGA;

(j) GHRVMVVVPRY;

(k) NDWHTALLPVYLKAYY;

(l) GIVNGIDNMEWNPEVD;

(m) DVPLLGFIGRLDGQKG;

(n) DVQLVMLGTG;

(o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(p)VGG(V/L)RDTV;

(xii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 37;

(xiii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 38; and

(xiv) a nucleotide sequence which is complementary to any one of (i) to (xiii).

12. An isolated or recombinant polypeptide, protein or enzyme comprising an amino acid sequence selected from the following:

(i) the amino acid sequence set forth in SEQ ID NO: 2 or the mature protein region thereof;

(ii) the amino acid sequence set forth in SEQ ID NO: 4 or the mature protein region thereof;

(iii) the amino acid sequence set forth in SEQ ID NO: 6 or the mature protein region thereof;

(iv) the amino acid sequence set forth in SEQ ID NO: 8 or the mature protein region thereof;

(v) the amino acid sequence set forth in SEQ ID NO: 10 or the mature protein region thereof;

(vi) a wheat starch synthase polypeptide having at least about 85% identity overall to any one of (i) to (v).

13. The isolated or recombinant polypeptide according to claim 12 further comprising one or more amino acid sequences selected from the group consisting of:

(a) KVGGLGDVVTS;

(b) GHTVEVILPKY;

(c) HDWSSAPVAWLYKEHY;

(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVVLLGSA;

(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS;

(h)TGGLVDTV;

(i) KTGGLGDVAGA;

(j) GHRVMVVVPRY;

(k) NDWHTALLPVYLKAYY;

(l) GIVNGIDNMEWNPEVD;

(m) DVPLLGFIGRLDGQKG;

(n) DVQLVMLGTG;

(o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(p)VGG(V/L)RDTV.

14. The isolated or recombinant polypeptide according to claim 13 wherein the wheat starch synthase polypeptide comprises at least three of said amino acid sequences selected from the group consisting of (a) to (h).

15. The isolated or recombinant polypeptide according to claim 13 wherein the wheat starch synthase polypeptide comprises at least six of said amino acid sequences selected from the group consisting of (i) to (p).

16. The isolated or recombinant polypeptide according to claim 12 encoding a wheat starch synthase II polypeptide.

17. The isolated or recombinant polypeptide according to claim 12 encoding a wheat starch synthase III polypeptide.

18. An isolated or recombinant starch synthase polypeptide which comprises one or more amino acid sequences selected from the group consisting of:

(a) GHTVEVILPKY;

(b) HDWSSAPVAWLYKEHY;

(c) DVPIVGIITRLTAQKG;

(d) NGQVVLLGSA;

(e)AGSDFIIVPSIFEPCGLTQLVAMRYGS;

(f)TGGLVDTV;

(g) GIVNGIDNMEWNPEVD; and

(h) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT.

19. The isolated or recombinant polypeptide according to claim 16 consisting of a wheat starch synthase II polypeptide which comprises an amino acid sequence selected from the group consisting of:

(i) SEQ ID NO: 2;

(ii) SEQ ID NO: 4;

(iii) SEQ ID NO: 6; and

(iv) a homologue of any one of (i) to (iii) having at least about 85% identity thereto.

20. The isolated or recombinant polypeptide according to claim 17 consisting of a wheat starch synthase III polypeptide which comprises an amino acid sequence selected from the group consisting of:

(i) SEQ ID NO: 8;

(ii) SEQ ID NO: 10; and

(iii) a homologue of (i) or (ii) having at least about 85% identity thereto.

21. The isolated or recombinant polypeptide according to claim 12 substantially free of conspecific or non-specific proteins.

22. A method comprising:

(i) hybridising single-stranded or double-stranded mRNA, cDNA or genomic DNA with a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence according to any one of claims 1 to 9;

(b) a probe or primer derived from a nucleotide sequence according to sub-paragraph (a) and comprising at least about 15 contiguous nucleotides of said nucleotide sequence in length; and

(ii) detecting the hybridised mRNA, cDNA or genomic DNA using a detecting means.

23. The method according to claim 22 wherein the detecting means consists of a reporter molecule covalently attached to the probe or primer molecule.

24. The method according to claim 22 wherein the detecting means consists of a polymerase chain reaction.

25. The method according to claim 22 wherein the probe or primer comprises a nucleotide sequence selected from the group consisting of:

(i) the nucleotide sequence set forth in SEQ ID NO: 25;

(ii) the nucleotide sequence set forth in SEQ ID NO: 26;

(iii) the nucleotide sequence set forth in SEQ ID NO: 27;

(iv) the nucleotide sequence set forth in SEQ ID NO: 28;

(v) the nucleotide sequence set forth in SEQ ID NO: 29;

(vi) the nucleotide sequence set forth in SEQ ID NO: 30;

(vii) the nucleotide sequence set forth in SEQ ID NO: 31;

(viii) the nucleotide sequence set forth in SEQ ID NO: 32;

(ix) the nucleotide sequence set forth in SEQ ID NO: 33;

(x) the nucleotide sequence set forth in SEQ ID NO: 34;

(xi) a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of:

(a) KVGGLGDVVTS;

(b) GHTVEVILPKY;

(c) HDWSSAPVAWLYKEHY;

(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVVLLGSA;

(g)AGSDFIIVPSIFEPCGLTQLVAMRYGS;

(h)TGGLVDTV;

(i) KTGGLGDVAGA;

(j) GHRVMVVVPRY;

(k) NDWHTALLPVYLKAYY;

(l) GIVNGIDNMEWNPEVD;

(m) DVPLLGFIGRLDGQKG;

(n) DVQLVMLGTG;

(o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(p)VGG(V/L)RDTV;

(xii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 37;

(xiii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 38; and

(xiv) a nucleotide sequence which is complementary to any one of (i) to (xiii).

26. A method of assaying for the presence or absence of a wheat starch synthase polypeptide in a plant or a plant extract or isolated nucleic acid sample, said method at least comprising performing the method according to any one of claims 22 to 25.

27. The method according to claim 26 further comprising preparing the plant extract or nucleic acid sample.

28. A method of marker-assisted breeding and/or selection of a plant at least comprising performing the method according to any one of claims 22 to 25.

29. The method according to claim 28 further comprising selecting a plant which expresses a desirable wheat starch synthase characteristic.

30. The method according to claim 28 further comprising crossing a plant which expresses a desirable wheat starch synthase characteristic to another plant.

31. The method according to claim 30 further comprising selecting progeny of the cross

which expresses a desirable wheat starch synthase characteristic.

32. A plant produced by the method according to any one of claims 28 to 31 wherein said plant expresses a wheat starch synthase polypeptide at a desired level detectable using said method.

33. A method of modifying the starch content and/or starch composition of one or more tissues or organs of a plant, comprising expressing in said plant a nucleic acid molecule for a time and under conditions sufficient for the enzyme activity of one or more starch synthase isoenzymes to be modified, wherein said nucleic acid molecule is selected from the group consisting of:

(i) the isolated nucleic acid molecule according to any one of claims 1 to 9;

(ii) a fragment of (i) which comprises a nucleotide sequence capable of being expressed to down-regulate the expression of an endogenous wheat starch synthase isoenzyme of said plant; and

(iii) a fragment of (i) which encodes a functional wheat starch synthase isoenzyme of said plant.

34. The method according to claim 33 wherein the fragment at sub-paragraph (ii) is an antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule.

35. The method according to claim 33 further comprising introducing the nucleic acid molecule to an isolated plant cell, tissue, organ, or organelle.

36. The method according to claim 35 further comprising regenerating an intact plant from the isolated plant cell, tissue, organ, or organelle carrying the introduced nucleic acid molecule.

37. The method according to claim 35 wherein the nucleic acid molecule is introduced to the plant cell, tissue, organ, or organelle by introgression.

38. The method according to claim 35 wherein the nucleic acid molecule is introduced to the plant cell, tissue, organ, or organelle by transformation means.

39. An isolated promoter sequence comprising a nucleotide sequence selected from the group consisting of:

(i) nucleotides 1 to about 287 of SEQ ID NO: 11;

(ii) nucleotides 1 to about 1416 of SEQ ID NO: 37;

(iii) nucleotides 1 to about 973 of SEQ ID NO: 38;

(iv) a fragment of any one of (i) to (iii) capable of conferring expression on a heterologous gene in a monocotyledonous plant cell, tissue or organ; and

(v) a complementary nucleotide sequence to any one of (i) to (iv).

40. The isolated promoter sequence according to claim 39 that is operable in the endosperm.

41. A plant carrying the isolated nucleic acid molecule according to any one of claims 1 to 9 as an exogenous complement to its genome.

42. A progeny of the plant according to claim 41 wherein said progeny carries the introduced nucleic acid molecule.

43. A propagule of the plant according to claim 41 or 42 wherein said propagule carries the

introduced nucleic acid molecule present in said plant.

44. A gene construct or vector which comprises the isolated nucleic acid molecule according to any one of claims 1 to 9 and one or more origins of replication.

45. The gene construct according to claim 44 further comprising a promoter sequence in operable connection with said isolated nucleic acid molecule.

46. A gene construct or vector which comprises the probe or primer according to claim 10 or 11 and one or more origins of replication.

47. A modified starch derived from the plant according to claim 32 or 41 wherein said starch is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said plant.

48. A modified starch derived from the progeny according to claim 42 wherein said starch is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said progeny.

49. A modified starch derived from the propagule according to claim 43 wherein said starch is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said propagule.

50. A food product comprising the modified starch according to any one of claims 47 to 49.

51. The food product according to claim 50 consisting of flour or a flour-based food product.

52. The food product according to claim 50 or 51 selected from the group consisting of: flour-based sauce; leavened bread; unleavened bread; pasta, noodle; cereal; snack food; cake; and pastry.

53. Use of the modified starch according to any one of claims 47 to 49 in the preparation of a food product for consumption by an animal or human.

54. A modified protein derived from the plant according to claim 32 or 41 wherein said protein is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said plant.

55. A modified protein derived from the progeny according to claim 42 wherein said protein is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said progeny.

56. A modified protein derived from the propagule according to claim 43 wherein said protein is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said propagule.

57. A non-food product comprising the modified protein according to any one of claims 54 to 56.

58. The non-food product according to claim 57 selected from the group consisting of: films; coatings; adhesives; building materials; and packaging materials.

59. Use of the modified protein according to any one of claims 54 to 56 in the preparation of a non-food product.

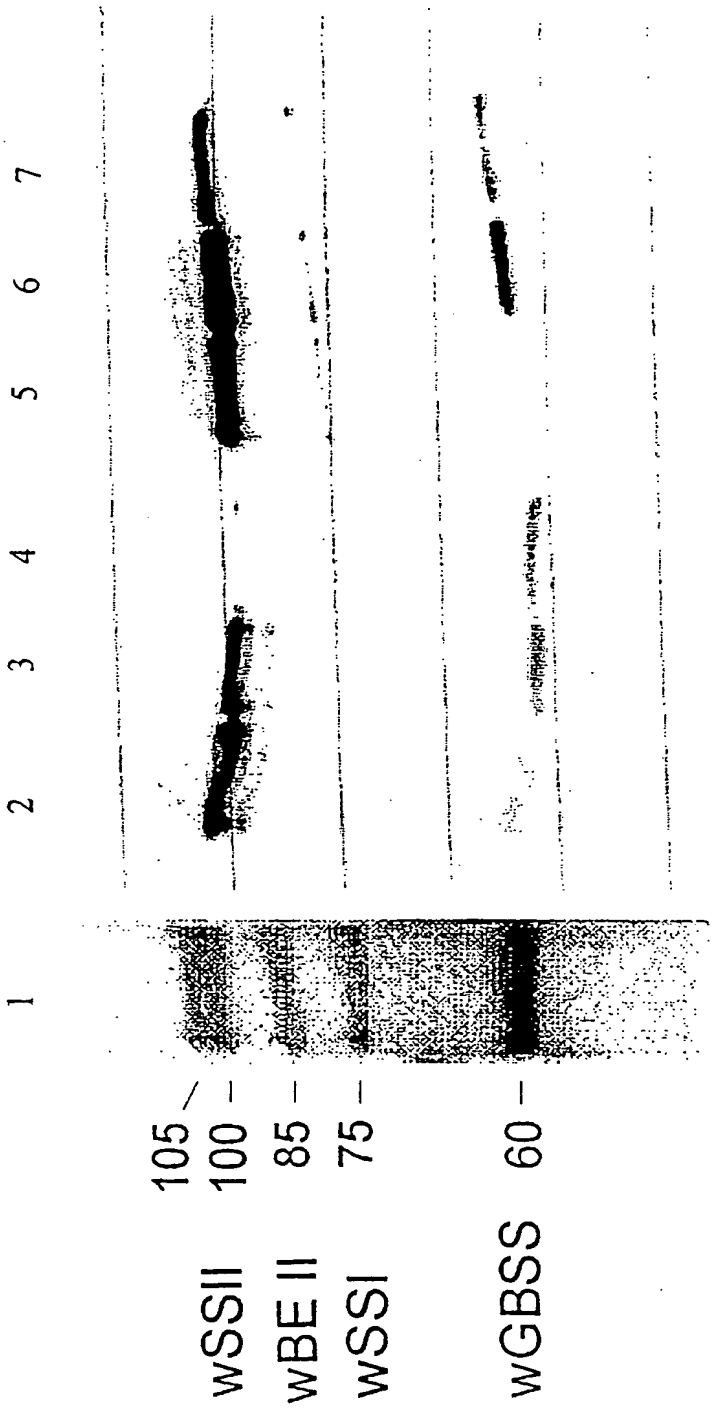


FIGURE 1

FIGURE 2A
FIGURE 2B
FIGURE 2C
FIGURE 2D
FIGURE 2E
FIGURE 2F
FIGURE 2G
FIGURE 2H
FIGURE 2I
FIGURE 2J
FIGURE 2K
FIGURE 2L
FIGURE 2M
FIGURE 2N
FIGURE 2O

FIGURE 2

```
          1                                                    50
wSSIIB    ATTTCCTCGG CCTGACCCCG TGCGTTTACC CCACACAGAG CACACTCCAG
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

          51                                                  100
wSSIIB    TCCAGTCCAG CCCACTGCCG CGCTACTCCC CACTCCCACT GCCACCACCT
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~GCT GCCACCACCT

          101                                                 150
wSSIIB    CCGCcTGCGC CGCGCTCTGG GCGGACCAAC CCGCGCATCG TATCACGATC
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    CCGCCTGCGC CGCGCTCTGG GCGGAGGACC AACCCGCGCA TCGTACCATC

          151                                                 200
wSSIIB    ACCCACCCCG ATCCCGGCCG CCGCCATGTC GTCGGCGGTC GCGTCCGCCG
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    GCCCGCCCCG ATCCCGGCCG CCGCCATGTC GTCGGCGGTC GCGTCCGCCG
```

FIGURE 2A

```
          201                                                        250
wSSIIB    CGTCCTTCCT CGCGCTCGCG TCCGCCTCCC CCGGGAGATC ACGGAGGAGG
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    CGTCCTTCCT CGCGCTCGCC TCCGCCTCCC CCGGGAGATC ACGCAGGCGG

          251                                                        300
wSSIIB    ACGAGGGTGA GCGCGTCGCC ACCCCACACC GGGGCTGGCA GGTTGCACTG
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    GCGAGGGTGA GCGCGCCGCC ACCCCACGCC GGGGCCGGCA GGCTGCACTG

          301                                                        350
wSSIIB    GCCGCCGTCG CCGCCGCAGC GCACGGCTCG CGACGGAGCG GTGGCCGCGC
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    GCCGCCGTGG CCGCCGCAGC GCACGGCTCG CGACGGAGGT GTGGCCGCGC

          351                                                        400
wSSIIB    GCGCCGCCGG GAAGAAGGAC GCGGGGAT.. .CGACGACGC CGCGCCCGCG
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    GCGCCGCCGG GAAGAAGGAC GCGAGGGTCG ACGACGACGC CGCGTCCGCG
```

FIGURE 2B

```
          401                                                        450
wSSIIB    AGGCAGCCCC GCGCACTCCG CGGTGGCGCC GCCACCAAGG TTGCGGAGCG
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    AGGCAGCCCC GCGCACGCCG CGGTGGCGCC GcCACCAAGG TCGCGGAGCG

          451                                                        500
wSSIIB    GAGGGATCCC GTCAAGACGC TCGATCGCGA CGCCGCGGAA GGTGGCGCGC
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    GAGGGATCCC GTCAAGACGC TCGATCGCGA CGCCGCGGAA GGTGGCGCGC

          501                                                        550
wSSIIB    CGTCCCCGCC GGCACCGAGG CAGGAGGACG CCCGTCTGCC GAGCATGAAC
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    CGGCACCGCC GGCACCGAGG CAGGACGCCG CCCGTCCaCC GAGTATGAAC

          551                                                        600
wSSIIB    GGCATGCCGG TGAACGGTGA AAACAAATCT ACCGGCGGCG GCGGCGCGAC
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    GGCACGCCGG TGAACGGTGA GAACAAATCT ACCGGCGGCG GCGGCGCGAC
```

FIGURE 2C

```
          601                                                        650
wSSIIB    TAAAGACAGC GGGCTGCCCG CACCCGCACG CGCGCCCCAG CCGTCGAGCC
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    cAAAGACAGC GGGCTgcCCG CACCCGcACG CGCGCCCCAT cCGTCGAcCC

          651                                                        700
wSSIIB    AGAACAGAGT ACCGGTGAAT GGTGAAAACA AAGCTAACGT CGCCTCGCCG
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    AgAACAgAGT ACCAGTGAAC GGTGAAAACA AAGCTAACGT CGCCTCGCCG

          701                                                        750
wSSIIB    CCGACGAGCA TAGCCGAGGT CGCGGCTCCG GATCCCGCAG CTACCATTTC
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    CCGACGAGCA TAGCCGAGGT CGTGGCTCCG GATTCCGCAG CTACCATTTC

          751                                                        800
wSSIIB    CATCAGTGAC AAGGCGCCAG AGTCCGTTGT CCCAGCCGAG AAGGcgccgc
wSSIID    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~CCAGCTGAG AAGACGCCGC
wSSIIA    CATCAGTGAC AAGGCGCCGG AGTCCGTTGT CCCAGCCGAG AAGCCGCCGC
```

FIGURE 2D

```
801                                                850
wSSIIB  CGtCgtcCgg CtcAAATtTc gtgCcCtCgg cttctGctCc cggGtctGAC
wSSIID  CGTCGTCCGG CTCAAATTTC GAGTCCTCGG CCTCTGCTCC CGGGTCTGAC
wSSIIA  CGTCGTCCGG CTCAAATTTC GTGgTCTCGG CTTCTGCTCC CAGGCTGGAC

        851                                        900
wSSIIB  actgtCaGCG acGtGGaact TgaActGAAg aAGGGtgCgg tCattgTcaA
wSSIID  ACTGTCAGCG ACGTGGAACA AGAACTGAAG AAGGGTGCGG TCGTTGTCGA
wSSIIA  ATTGACAGCG ATGTTGAACC TGAACTGAAG AAGGGTGCGG TCATCGTCGA

        901                                        950
wSSIIB  aGAAgcTcCa aaCcCaAaGG CTCTTTCGCC GCCCGCAGCA CCCGCTGTAC
wSSIID  AGAAGCTCCA AAGCCAAAGG CTCTTTCGCC GCCtGCAGCc CCCGCTGTAC
wSSIIA  AGAAGCTCCA AACCCAAAGG CTCTTTCGCC GCCTGCAGCC CCCGCTGTAC

        951                                       1000
wSSIIB  AACAAGACCT TTGGGACTTC AAGAAATACA TTGGTTTCGA GGAGCCCGTG
wSSIID  AAgAAGACCT TTGGGAtTTC AAGAAATACA TTGGTTTCGA GGAGCCCGTG
wSSIIA  AAGAAGACCT TTGGGACTTC AAGAAATACA TTGGCTTCGA GGAGCCCGTG
```

FIGURE 2E

```
          1001                                                      1050
wSSIIB    GAGGCCAAGG ATGATGGCCG GGCTGTTGCA GATGATGCGG GCTCCTTCGA
wSSIID    GAGGCCAAGG ATGATGGCCG GGCTGTcGCA GATGATGCGG GCTCCTTtGA
wSSIIA    GAGGCCAAGG ATGATGGCTG GGCTGTTGCA GATGATGCGG GCTCCTTTGA

          1051                                                      1100
wSSIIB    ACACCACCAG AATCACGATT CCGGGCCTTT GGCAGGGGAG AACGTCATGA
wSSIID    ACACCACCAG AATCACGAcT CCGGaCCTTT GGCAGGGGAG AAtGTCATGA
wSSIIA    ACATCACCAG AACCATGATT CCGGACCTTT GGCAGGGGAG AACGTCATGA

          1101                                                      1150
wSSIIB    ACGTGGTCGT CGTGGCTGCT GAATGTTCTC CCTGGTGCAA AACAGGTGGT
wSSIID    ACGTGGTCGT CGTGGCTGCT GAgTGTTCTC CCTGGTGCAA AACAGGTGGT
wSSIIA    ACGTGGTCGT CGTGGCTGCT GAATGTTCTC CCTGGTGCAA AACAGGTGGT

          1151                                                      1200
wSSIIB    CTTGGAGATG TTGCCGGTGC TTTGCCCAAG GCTTTGGCGA AGAGAGGACA
wSSIID    CTgGGAGATG TTGCgGGTGC TcTGCCCAAG GCTTTGGCaA AGAGAGGACA
wSSIIA    CTTGGAGATG TTGCCGGTGC TTTGCCCAAG GCTTTGGCGA AGAGAGGACA
```

FIGURE 2F

```
        1201                                                        1250
wSSIIB  TCGTGTTATG GTTGTGGTAC CAAGGTATGG GGACTATGAG GAAGCCTACG
wSSIID  TCGTGTTATG GTTGTGGTAC CAAGGTATGG GGACTATGAa GAACCTACGg
wSSIIA  TCGTGTTATG GTTGTGGTAC CAAGGTATGG GGACTATGAG GAAGCCTACG

        1251                                                        1300
wSSIIB  ATGTCGGAGT CCGAAAATAC TACAAGGCTG CTGGACAGGA TATGGAAGTG
wSSIID  ATGTCGGAGT CCGAAAATAC TACAAGGCTG CTGGACAGGA TATGGAAGTG
wSSIIA  ATGTCGGAGT CCGAAAATAC TACAAGGCTG CTGGACAGGA TATGGAAGTG

        1301                                                        1350
wSSIIB  AATTATTTCC ATGCTTATAT CGATGGAGTT GATTTTGTGT TCATTGACGC
wSSIID  AATTATTTCC ATGCTTaTAT CGATGGAGTT GATTTTGTGT TCATTGACGC
wSSIIA  AATTATTTCC ATGCTTATAT CGATGGAGTT GATTTTGTGT TCATTGACGC

        1351                                                        1400
wSSIIB  TCCTCTCTTC CGACACCGCC AGGAAGACAT TTATGGGGGC AGCAGACAGG
wSSIID  TCCTCTCTTC CGACACCGAG AGGAAGACAT TTATGGGGGC AGCAGACAGG
wSSIIA  TCCTCTCTTC CGACACCGCC AGGAAGACAT TTATGGGGGC AGCAGACAGG
```

FIGURE 2G

```
        1401                                                    1450
wSSIIB  AAATTATGAA GCGCATGATT TTGTTCTGCA AGGCCGCTGT CGAGGTTCCA
wSSIID  AAATTATGAA GCGCATGATT TTGTTCTGCA AGGCCGCTGT TGAGGTTCCA
wSSIIA  AAATTATGAA GCGCATGATT TTGTTCTGCA AGGCCGCTGT CGAGGTTCCT

        1451                                                    1500
wSSIIB  TGGCACGTTC CATGCGGCGG TGTCCCTTAT GGGGATGGAA ATCTGGTGTT
wSSIID  TGGCACGTTC CATGCGGCGG TGTCCCTTAT GGGGATGGAA ATCTGGTGTT
wSSIIA  TGGCACGTTC CATGCGGCGG TGTCCCTTAT GGGGATGGAA ATCTGGTGTT

        1501                                                    1550
wSSIIB  TATTGCAAAT GATTGGCACA CGGCACTCCT GCCTGTCTAT CTGAAAGCAT
wSSIID  TATTGCAAAT GATTGGCACA CGGCACTCCT GCCTGTCTAT CTGAAAGCAT
wSSIIA  TATTGCAAAT GATTGGCACA CGGCACTCCT GCCTGTCTAT CTGAAAGCAT

        1551                                                    1600
wSSIIB  ATTACAGGGA CCATGGTTTG ATGCAGTACA CTCGGTCCAT TATGGTGATA
wSSIID  ATTACAGGGA CCATGGTTTG ATGCAGTACA CTCGGTCCAT TATGGTGATA
wSSIIA  ATTACAGGGA CCATGGTTTG ATGCAGTACA CTCGGTCCAT TATGGTGATA
```

FIGURE 2H

```
        1601                                                        1650
wSSIIB  CATAACATCG CTCACCAGGG CCGTGGCCCA GTAGATGAGT TCCCGTTCAC
wSSIID  CATAACATCG CTCACCAGGG CCGTGGCCCT GTAGATGAAT TCCCGTTCAC
wSSIIA  CATAACATCG CGCACCAGGG CCGTGGCCCA GTAGATGAAT TCCCGTTCAC

        1651                                                        1700
wSSIIB  CGAGTTGCCT GAGCACTACC TGGAACACTT CAGACTGTAC GACCCCGTGG
wSSIID  CGAGTTGCCT GAGCACTACC TGGAACACTT CAGACTGTAC GACCCCGTGG
wSSIIA  CGAGTTGCCT GAGCACTACC TGGAACACTT CAGACTGTAC GACCCCGTGG

        1701                                                        1750
wSSIIB  GTGGTGAACA CGCCAACTAC TTCGCCGCCG GCCTGAAGAT GGCGGACCAG
wSSIID  GTGGTGAACA CGCCAACTAC TTCGCCGCCG GCCTGAAGAT GGCGGACCAG
wSSIIA  GTGGTGAGCA CGCCAACTAC TTCGCCGCCG GCCTgAAGAT GgCGGACCAG

        1751                                                        1800
wSSIIB  GTTGTCGTCG TGAGCCCGGG GTACCTGTGG GAGCTGAAGA CGGTGGAGGG
wSSIID  GTTGTCGTGG TGAGCCCCGG GTACCTGTGG GAGCTGAAGA CGGTGGAGGG
wSSIIA  GTTGTCGTGG TGAGCCCCGG GTACCTGTGG gAGCTCAAGA CGGTGGAgGG
```

FIGURE 2I

```
          1801                                                      1850
wSSIIB    CGGCTGGGGG CTTCACGACA TCATACGGCA GAACGACTGG AAGACCCGCG
wSSIID    CGGCTGGGGG CTTCACGACA TCATACGGCA GAACGACTGG AAGACCCGCG
wSSIIA    CGGCTGGGGG CTTCACGACA TCATACGGCA GAACGACTGG AAGACCCGCG

          1851                                                      1900
wSSIIB    GCATCGTGAA CGGCATCGAC AACATGGAGT GGAACCCCGA GGTGGACGTC
wSSIID    GCATCGTCAA CGGCATCGAC AACATGGAGT GGAACCCCGA GGTGGACGCC
wSSIIA    GCATCGTCAA CGGCATCGAC AACATGGAGT GGAACCCCGA GGTGGACGTC

          1901                                                      1950
wSSIIB    CACCTCAAGT CGGACGGCTA CACCAACTTC TCCCTGGGGA CGCTGGACTC
wSSIID    CACCTCAAGT CGGACGGCTA CACCAACTTC TCCCTGAGGA CGCTGGACTC
wSSIIA    CACCTCAAGT CGGACGGCTA CACCAACTTC TCCCTGGGGA CGCTGGACTC

          1951                                                      2000
wSSIIB    CGGCAAGCGG CAGTGCAAGG AGGCCCTGCA GCGGGAGCTG GGCCTGCAGG
wSSIID    CGGCAAGCGG CAGTGCAAGG AGGCCCTGCA GCGCGAGCTG GGCCTGCAGG
wSSIIA    CGGCAAGCGG CAGTGCAAGG AGGCCCTGCA GCGCGAGCTG GGCCTGCAGG
```

FIGURE 2J

```
          2001                                                      2050
wSSIIB    TCCGCGGCGA CGTGCCGCTG CTCGGCTTCA TCGGGCGCCT GGACGGGCAG
wSSIID    TCCGCGCCGA CGTGCCGCTG CTCGGCTTCA TCGGCCGCCT GGACGGGCAG
wSSIIA    TCCGCGCCGA CGTGCCGCTG CTCGGCTTCA TCGGCCGCCT GGACGGGCAG

          2051                                                      2100
wSSIIB    AAGGGCGTGG AGATCATCGC GGACGCGATG CCCTGGATCG TGAGCCAGGA
wSSIID    AAGGGCGTGG AGATCATCGC GGACGCCATG CCCTGGATCG TGAGCCAGGA
wSSIIA    AAGGGCGTGG AGATCATCGC GGACGCCATG CCCTGGaTCG TGAGCCAGgA

          2101                                                      2150
wSSIIB    CGTGCAGCTG GTCATGCTGG GCACCGGGCG CCACGACCTG GAGGGCATGC
wSSIID    CGTGCAGCTG GTGATGCTGG GCACCGGGCG CCACGACCTG GAGAGCATGC
wSSIIA    CGTGCAGCTG GTCATGCTGG GCACCGGCCG CCACGACcTG gAGAGCATGc

          2151                                                      2200
wSSIIB    TGCGGCACTT CGAGCGGGAG CACCACGACA AGGTGCGCGG GTGGGTGGGG
wSSIID    TGCAGCACTT CGAGCGGGAG CACCACGACA AGGTGCGCGG GTGGGTGGGG
wSSIIA    TgCGGCACTT CGAGCGGGAG CACCACGACA AGGTGCGCGG gTGGGTGGGG
```

FIGURE 2K

```
     2201                                                        2250
wSSIIB  TTCTCCGTGC GGCTGGCGCA CCGGATCACG GCCGGCGCCG ACGCGCTCCT
wSSIID  TTCTCCGTGC GCCTGGCGCA CCGGATCACG GCGGGGGCGG ACGCGCTCCT
wSSIIA  TTCTCCGTgc GccTGGCGCA CCGGATCACG GCGGGCGCCG ACGCGCTCcT

        2251                                                    2300
wSSIIB  CATGCCCTCC CGGTTCGAGC CGTGCGGACT GAACCAGCTC TACGCCATGG
wSSIID  CATGCCCTCC CGGTTCGTGC CGTGCGGGCT GAACCAGCTC TACGCCATGG
wSSIIA  CATGCCCTCC CGGTTCGAgC CGTGCGGGTT GAACCAGCTt TACGCCATGG

        2301                                                    2350
wSSIIB  CCTACGGCAC CGTCCCCGTC GTGCATGCCG TCGGTGGCCT GAGGGACACC
wSSIID  CCTACGGCAC CGTCCCCGTC GTGCACGCCG TCGGCGGCCT CAGGGACACC
wSSIIA  CCTACGGCAC CGTCCCCGTC GTGCACGCCG TCGGCGGGGT GAGGGACACC

        2351                                                    2400
wSSIIB  GTGCCGCCGT TCGACCCCTT CAACCACTCC GGGCTCGGGT GGACGTTCGA
wSSIID  GTGCCGCCGT TCGACCCCTT CAACCACTCC GGGCTCGGGT GGACGTTCGA
wSSIIA  GTGCCGCCGT TCGACCCCTT CAACCACTCC GGcCTCGGGT GGACGTTCGA
```

FIGURE 2L

```
          2401                                                      2450
wSSIIB    CCGCGCAGAG GCGCAGAAGC TGATCGAGGC GCTCGGGCAC TGCCTCCGCA
wSSIID    CCGCGCCGAG GCGCACAAGC TGATCGAGGC GCTCGGGCAC TGCCTCCGCA
wSSIIA    CCGCGCcGAG GCGCAcAAGC TGATCGAGGC GCTCGGGCAC TGCCTCCGCA

          2451                                                      2500
wSSIIB    CCTACCGGGA CTACAAGGAG AGCTGGAGGG GGCTCCAGGA GCGCGGCATG
wSSIID    CCTACCGAGA CTTCAAGGAG AGCTGGAGGG CCCTCCAGGA GCGCGGCATG
wSSIIA    CCTACCGGGA CTACAAGGAG AGCTGGAGGG GcCTCCAGGA GCGCGGCATG

          2501                                                      2550
wSSIIB    TCGCAGGACT TCAGCTGGGA GCATGCCGCC AAGCTCTACG AGGACGTCCT
wSSIID    TCGCAGGACT TCAGCTGGGA GCACGCCGCC AAGCTCTACG AGGACGTCCT
wSSIIA    TCGCAGGACT TCAGCTGGGA GCATGCCGCC AAGCTCTACG AGGACGTCCT

          2551                                                      2600
wSSIIB    CGTCAAGGCC AAGTACCAGT GGTGAACGCT AGCTGCTAGC CGGTCCAGCC
wSSIID    CGTCAAGGCC AAGTACCAGT GGTGAACGCT AGCTGCTAGC CGCTCCAGCC
wSSIIA    CcTCAAGGCC AAGTACCAGT GGTGAACGCT AGCTGCTAGC CGcTCCAGCC
```

FIGURE 2M

```
          2601                                                    2650
wSSIIB    CCGCATGCG. ...TGCATGA CAGGATGGAA TTGCGCATTG CGCACGCAGG
wSSIID    CCGCATGCG. ...TGCATGA CAGGATGGAA CT..GCATTG CGCACGCAGG
wSSIIA    CCGCATGCGT GCATGcatgA gAGGgTGGAA cTGCGCATTG CGCcCGCAGG

          2651                                                    2700
wSSIIB    AAGGTGCCAT .......... .GGAGCGCCG GCATCCGCGA AGTACAGTGA
wSSIID    AAAGTGCCAT .......... .GGAGCGCCG GCATCCGCGA AGTACAGTGA
wSSIIA    AAcGTGCCAT ccttctcgat gGGAGCGCCG GCATCCGCGA gGTgCAGTGA

          2701                                                    2750
wSSIIB    CAT..GAGGT GTGTGTGGTT GAGACGCTGA TTC......C GATCTGGTCC
wSSIID    CAT..GAGGT GTGTGTGGTT GAGACGCTGA TTC......C AATCCGGCCC
wSSIIA    CATGAGagGT GTGTGTGGTT GAGACGCTGA TTCCGATCTc gatctGGTCC

          2751                                                    2800
wSSIIB    GTAGCAGAGT AGAGCGGAGG TAGGGAAGCG CTCCTTGTTA CAGGTATATG
wSSIID    GTAGCAGAGT AGAGCGGAGG TATATGGGAA TCTTAACTTG GTATTGTAAT
wSSIIA    GTAGCAGAGT AGAGCGGAcG TAGGGAAGCG CTCCTTGTTg CAGGTATATG
```

FIGURE 2N

```
          2801                                                      2850
wSSIIB    GGAATGTTGT TAACTTGGTA TTGTAATTTG TTATGTTGTG TGCATTATTA
wSSIID    TTGTTATGTT GTGTGCATTA TTACAATGTT GTTACTTATT CTTGTTAAGT
wSSIIA    GGAATGTTGT cAACTTGGTA TTGTAgTTTG cTATGTTGTa TGCgTTATTA

          2851                                                      2900
wSSIIB    CAGAGGGCAA CGATCTGCGC CGGCGCACCG GCCCAACTGT TGGGCCGGTC
wSSIID    CGGAGGCCAA GGGCGAAAGC TAGCTCACAT GTCTGATGGA TGCAAAAAAA
wSSIIA    caatgttgtt acttattctt gtTAAAAAAA AAAAAAAAAA AAAA~~~~~~

          2901                                                      2950
wSSIIB    GCACAGCAGC CGTTGGATCC GACCGCCTGG GCCGTTGGAT CCCACCGAAA
wSSIID    AAAAAAAAAA AAA~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
wSSIIA    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

          2951       2965
wSSIIB    AAAAAAAAAA AAAAA
wSSIID    ~~~~~~~~~~ ~~~~~
wSSIIA    ~~~~~~~~~~ ~~~~~
```

FIGURE 2O

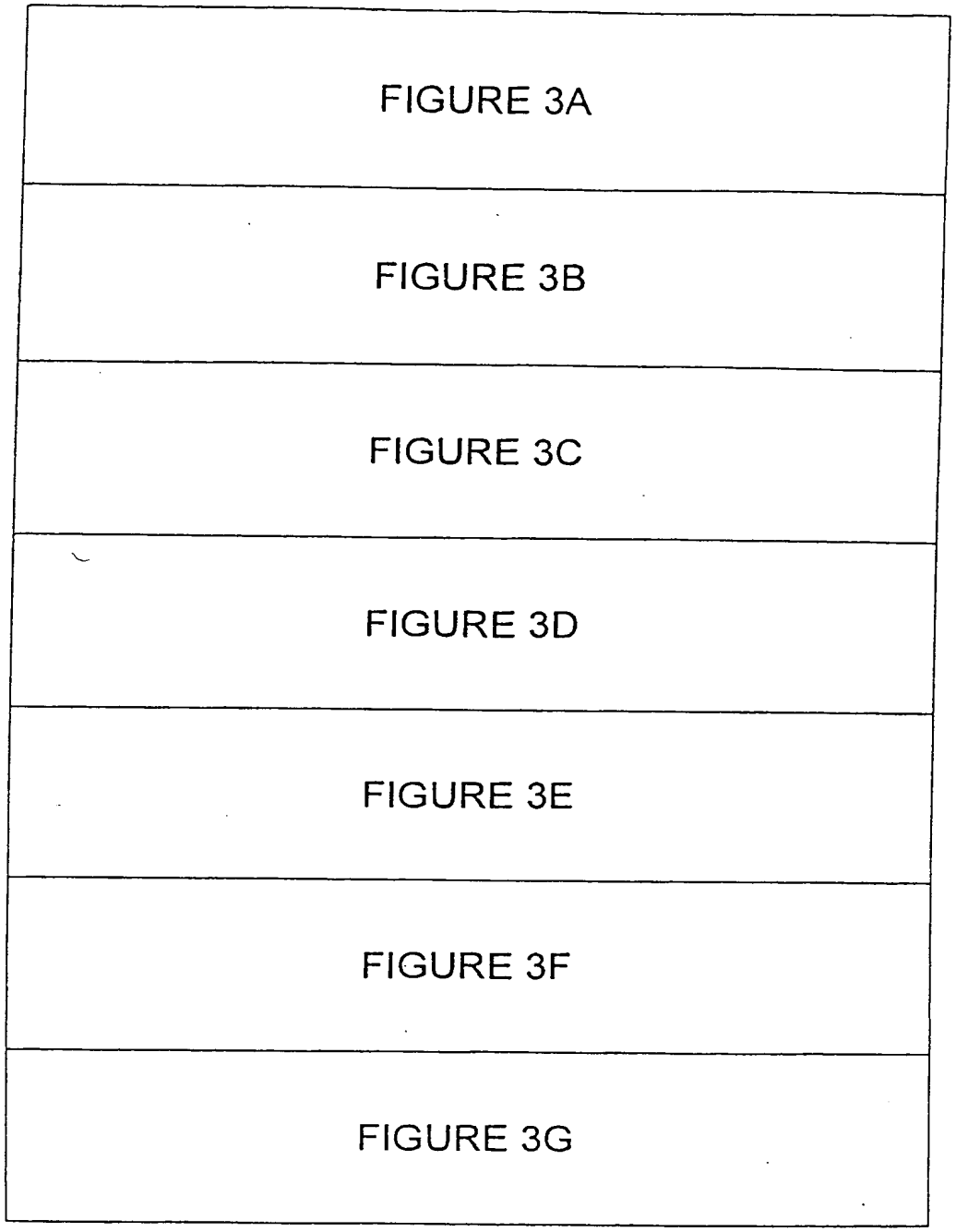


FIGURE 3A
FIGURE 3B
FIGURE 3C
FIGURE 3D
FIGURE 3E
FIGURE 3F
FIGURE 3G

FIGURE 3

```
WSSIIA    1 MSSAVASAAS ---FLALASA SP-GRSRRRA RVSAPPPHAG AGRL----HW PPWPP-QRTA  51
WSSIIB    1 ********** --******** **-******T ****S***T* ****-----** **S**-****  51
WSSIID      ---------- ---------- ---------- ---------- ---------- ----------
ZSSIIA    1 ****AV*SS* STF******* **G**--*** **GSS*F*T* *-S*SFAFWA **S**RAPRD  57
ZSSIIB    1 *PG*-I*SS* SAFL*PV**S **---R***G S*G*ALRSY* YSGAELRL** ARRG*P*DG*  56
PEASSII   1 *MLSLG*D*T VLP*H*KNLK FTPKL*TLNG --DLAFSKGL GVGRLNCGSV ----------R  49
POTSSII  10 PVNFIFCDFY VMENSI*LHS GNQFHPNLPL ---LALRPKK LSLIHGSSRE ----------Q  57

                    ⇓ Transit peptide cleavage site
WSSIIA.   52 RDGGVAARAA GKKDARVDDD AASARQPRAR RGGAATKVAE RRDPVKTLDR DAAEGGAPAP 111
WSSIIB    52 ***A****** *****GI-** **P******L ********** ********** ********S* 110
WSSIID       ---------- ---------- ---------- ---------- ---------- ----------
ZSSIIA    58 AALVR*EAE* *G***PPERS GDA**L**** *----NA*SK ****------ ----------  97
ZSSIIB    57 -ASVR**A*P AGG------- ---------- ---------- ---------- ----------  68
PEASSII   50 LNHKQHV**V **SFGADENG DG*EDDVVNA TIEKSK**LA LQRELIQQIA ERKKLVSSID 109
POTSSII   58 MWRNQRVK*T *ENSGEAA-S *DESNDALQV TIEKSK**LA MQQDLLQQIA ERRKVVSSIK 116
```

FIGURE 3A

```
WSSIIA  112 PAPRQDAARP PSMNGTPVNG ENKSTGGGGA TKDSGLPAPA RAPHPSTQNR VPVNGENKAN 171
WSSIIB  111 *****ED**L *****M**** ********** ********** ***Q**S*** ********** 170
WSSIID      ---------- ---------- ---------- ---------- ---------- ----------
ZSSIIA   98 ---------- ---------- -----LQPVG RYG*ATGNT* *TGAA*C**A ALADV*I*SI 132
ZSSIIB   69 ---------- ---------- ---------- -ESEEAAKSS SSSQAGAVQG STAKAVDS*S  97
PEASSII 110 SDSIPGLEGN GVSYESSEKS LSR------- ---------- ------DS*P QKGSSSSGSA 146
POTSSII 117 S----SL*NA KGTYDGGSGS LSDVDIPDVD KDYNVTVPST A*TGITDVDK NTPPAISHDF 172

WSSIIA  172 VASPPTSIAE VVAPDSAATI SISDKAPESV VPAEKPPPSS GSNFVVSASA PRLDIDSDVE 231
WSSIIB  171 ********** *A***P**** ********** *****A**** *****P**** *GS*TV**** 230
WSSIID  203 ---------- ---------- ---------- -****T**** ****ES**** *GS*TV**** 231
ZSSIIA  134 **A*****VK FP**GYRMIL PSG*I***T* L**P**--LH E*PA*DGD*N --GIAPPT** 188
ZSSIIB   99 PPN*L**APK QSQSAAMQNG TSGGSSASTA A*VSG*KADH P*AP*TKREI DASAVKPEPA 158
PEASSII 147 *ETKR--WHC FQQ-----LC RSKETETWA* SSVGINQGFD EIEKKND*VK ASSKLHFNEQ 199
POTSSII 173 *E*KREIKRD LADERAPPLS RS*IT*SSQI SSTVSSK--R TL*VPPETPK SSQETLL**N 230
```

FIGURE 3B

```
                                                                wSSIIp1 Region
WSSIIA   232 PELKKGAVIV EEAPNPKALS PPAAPAVQED LWDFKKYIGF EEPVEAKDDG WAVADDAGSF 291
WSSIIB   231 L********* K********* ********Q* ********** ********** R********* 290
WSSIID   232 Q*******V* ****K***** ********** ********** ********** R********* 291
ZSSIIA   189 *---------- ---------- -----L***A T********* D**D*****S RVG******* 224
ZSSIIB   159 GDDARPVESI ---------- ---------- --------*I A***D**A*- A*P*T**AAS 188
PEASSII  200 IKN*LYERPD TKDIS--SSI R--------- ------TSSL KFENFEGANE PSSKEV*NEA 242
POTSSII  231 SRKSLVD*PG KKIQSYMPSL R--------- ----*ESSAS HVEQRNENLE GSS*EANEET 277

                                                    Region 1                Region 2
WSSIIA.  292 EHHQNHD--S GPLAGENVMN VVVVAAECSP WCKTGGLGDV AGALPKALAK RGHRVMVVVP 349
WSSIIB   291 ********--* ********** ********** ********** ********** ********** 348
WSSIID   292 ********--* ********** ********** ********** ********** ********** 349
ZSSIIA   225 **YGDN*--* ********** *I******** ********** V********R ********** 282
ZSSIIB   189 APYDRE*NEP *****P**** *****S**A* F********* V********R ********I* 248
PEASSII  243 *NFESGGEKP P****T**** IIL*S***A* *S******** **S******R ******I*A* 302
POTSSII  278 *DPV*I*EKP P****T**** IIL**S**A* *S******** *********R ********A* 337
```

FIGURE 3C

Sgp-1 Peptide 3

```
WSSIIA  350 RYGDYEEAYD VGVRKYYKAA GQDMEVNYFH AYIDGVDFVF IDAPLFRHRQ EDIYGGSRQE 409
WSSIIB  349 ********** ********** ********** ********** ********** ********** 409
WSSIID  350 *******PT* ********** ********** ********** *********E ********** 408
ZSSIIA  283 *****V**F* **I******* ***L****** *F********* ********** D********* 409
ZSSIIB  249 ***E*A**R* L***RR**V* ***S**T*** S********* VE**P****H NN****E*LD 342
PEASSII 303 H**N*A**H* I****R**V* ******T*** T******I** **S*I**NLE SN****N*LD 308
POTSSII 338 **DN*P*PQ* S****I**VD ***VD*T**Q *LLMDC**** *HSHM***IG NN****N*VD 397
```

Region 3

```
WSSIIA  410 IMKRMILFCK AAVEVPWHVP CGGVPYGDGN LVFIANDWHT ALLPVYLKAY YRDHGLMQYT 469
WSSIIB  409 ********** ********** ********** ********** ********** ********** 469
WSSIID  410 ********** ********** ********** ********** ********** ********** 468
ZSSIIA  343 ********** V********* ****C***** ********** ********** ********** 469
ZSSIIB  309 *L******** *******YA* ***TV***** ********** ********** ********** 402
PEASSII 363 *LR**V**** ********** ***IC***** ********** ********** ***N*****A 368
POTSSII 398 *L***V**** **I******* ****C***** ********** ****A***** ***N*I*N** 457
```

FIGURE 3D

```
WSSIIA   470 RSIMVIHNIA HQGRGPVDEF PFTELPEHYL EHFRLYDPVG GEHANYFAAG LKMADQVVVV 529
WSSIIB   469 ********** ********** ********** ********** ********** ********** 528
WSSIID   470 ********** ********** ********** ********** ********** ********** 529
ZSSIIA   404 **VL****** ********** *YMD****** Q**E****** *****I**** *****R**T* 462
ZSSIIB   369 **VL****** ********D* VNFD*****I D**K***NI* *D*S*V**** **T**R**T* 428
PEASSII  423 **VL****** *******ED* NTVD*SGN** DL*KM***** ***F*I**** **T**RI*T* 482
POTSSII  458 **VL****** ******LED* SYVD**P**M DP*K****** ***F*I**** **T**R**T* 517

                                    Region 4
WSSIIA   530 SPGYLWELKT VEGGWGLHDI IRQNDWKTRG IVNGIDNMEW NPEVDVHLK- SDGYTNFSLG 588
WSSIIB   529 ********** ********** ********** ********** ********** ********** 587
WSSIID   530 ********** ********** ********** ********** *****A***- ********** 588
ZSSIIA   463 *R******** ********** **S****IN* ******HQ** **K*****R- ******Y**E 521
ZSSIIB   429 *N**M***** S********* *N*****LQ* ******MS** **A*****H- **D***YTFE 487
PEASSII  483 *H**A***** S*******N* *NES***F** ****V*TKD* **QF*AY*T- ******YN*K 541
POTSSII  518 *H**S***** SQ******Q* *NE****LQ* ******TK** ***L****PR ****M*Y**D 577
```

FIGURE 3E

```
                                                  Region 5                                                  Region 5a
WSSIIA   589 TLDSGKRQCK EALQRELGLQ VRADVPLLGF IGRLDGQKGV EIIADAMPWI VSQDVQLVML 648
WSSIIB   588 ********** ********** **G******* ********** ********** ********** 647
WSSIID   588 ********** ********** ********** ********** ********** ********** 648
ZSSIIA   522 ***A****** A********E **D******* ********** D**G****** AG******** 581
ZSSIIB   488 ***T****** A****Q**** **D****I** *****H**** D*****IH** AG******** 547
PEASSII  542 **QT****** A********P **E***IIS* *****H**** DL**E*I**M M*H******* 601
POTSSII  578 **QT**P*** A***K****P **D****I** *****P**** DL**E*V**M MG******** 637

                                                  Region 6
WSSIIA   649 GTGRHDLESM LRHFEREHHD KVRGWVGFSV RLAHRITAGA DALLMPSRFE PCGLNQLYAM 708
WSSIIB   648 ********G* ********** ********** ********** ********** ********** 707
WSSIID   649 ********** *Q******** ********** ********** *********V ********** 708
ZSSIIA   582 ****A***R* *Q*L****PN ********** PM******** *V*V****** ********** 641
ZSSIIB   548 ****A***D* **R**S**S* ***A****** P********* *I******** ********** 607
PEASSII  602 ****A***Q* *KE**AQ*C* *I*S****** KM*******S *I******** ********** 661
POTSSII  638 ****R***Q* **Q**CQ*N* *I******** KTS******** *I******** **A******* 697
```

FIGURE 3F

```
                        Region 7
WSSIIA   709 AYGTVPVVHA VGGVRDTVPP FDPFNHSGLG WTFDRAEAHK LIEALGHCLR TYRDYKESWR 768
WSSIIB   708 ********** ***L****** ********** ********Q* ********** ********** 767
WSSIID   709 ********** ***L****** ********** ********** ********** ****F***** 768
ZSSIIA   642 ********** ***L****A* ****GDA*** ********N* *****R***D ***K*G***K 701
ZSSIIB   608 ********** ***L****A* *****DT*** ********NR M*D**S***T ***N****** 667
PEASSII  662 S********G ***L****Q* *N**DE**V* ********N* *MA**WN**L **K***K**E 721
POTSSII  698 K***I***** ***L****Q* ***LMSQDW* GPS*****SQ **PRIRN**L ***E**K**E 757

WSSIIA   769 GLQERGMSQD FSWEHAAKLY EDVLLKAKYQ W 799
WSSIIB   768 ********** ********** ****V***** * 798
WSSIID   769 ********** ********** ****V***** * 799
ZSSIIA   702 S**A****** L**D***E** ****V***** * 732
ZSSIIB   668 ACRA***AE* L**D***V** ****V***** * 698
PEASSII  722 *I******** L**DN**QQ* *E**VA**** * 752
POTSSII  759 *I*T*C*T** L**DN**QN* *E**IA**** * 788
```

FIGURE 3G

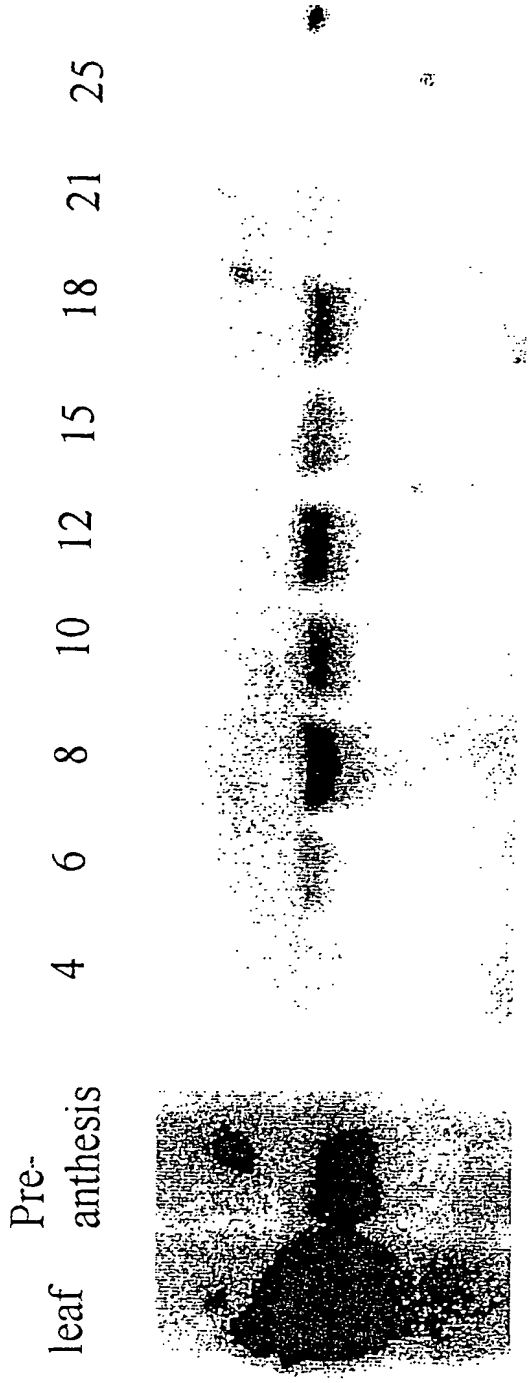


FIGURE 4

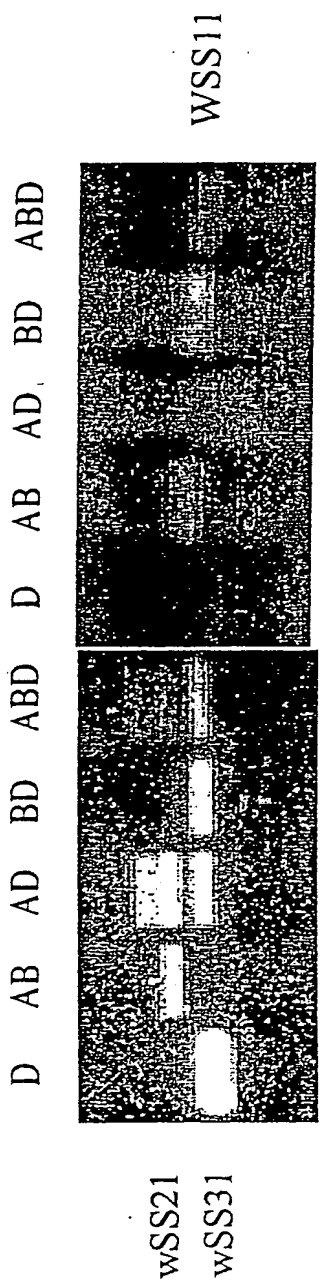


FIGURE 5

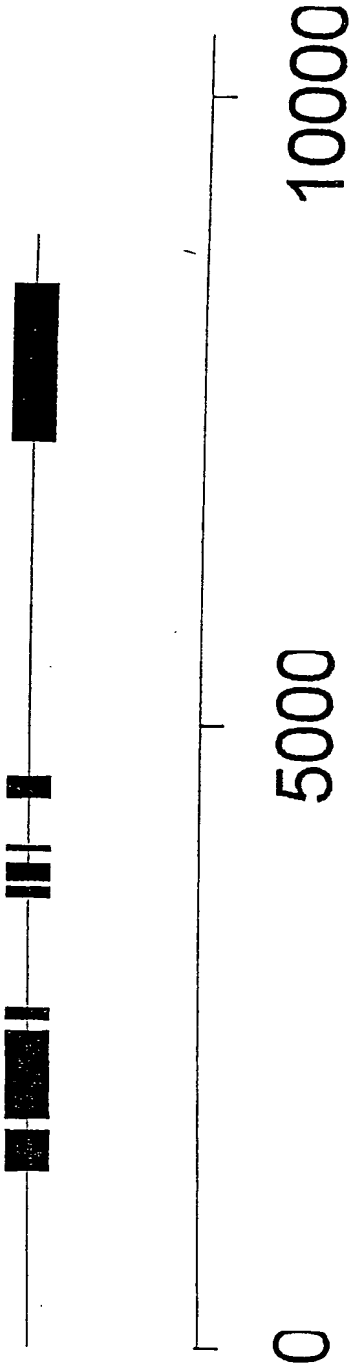


FIGURE 6

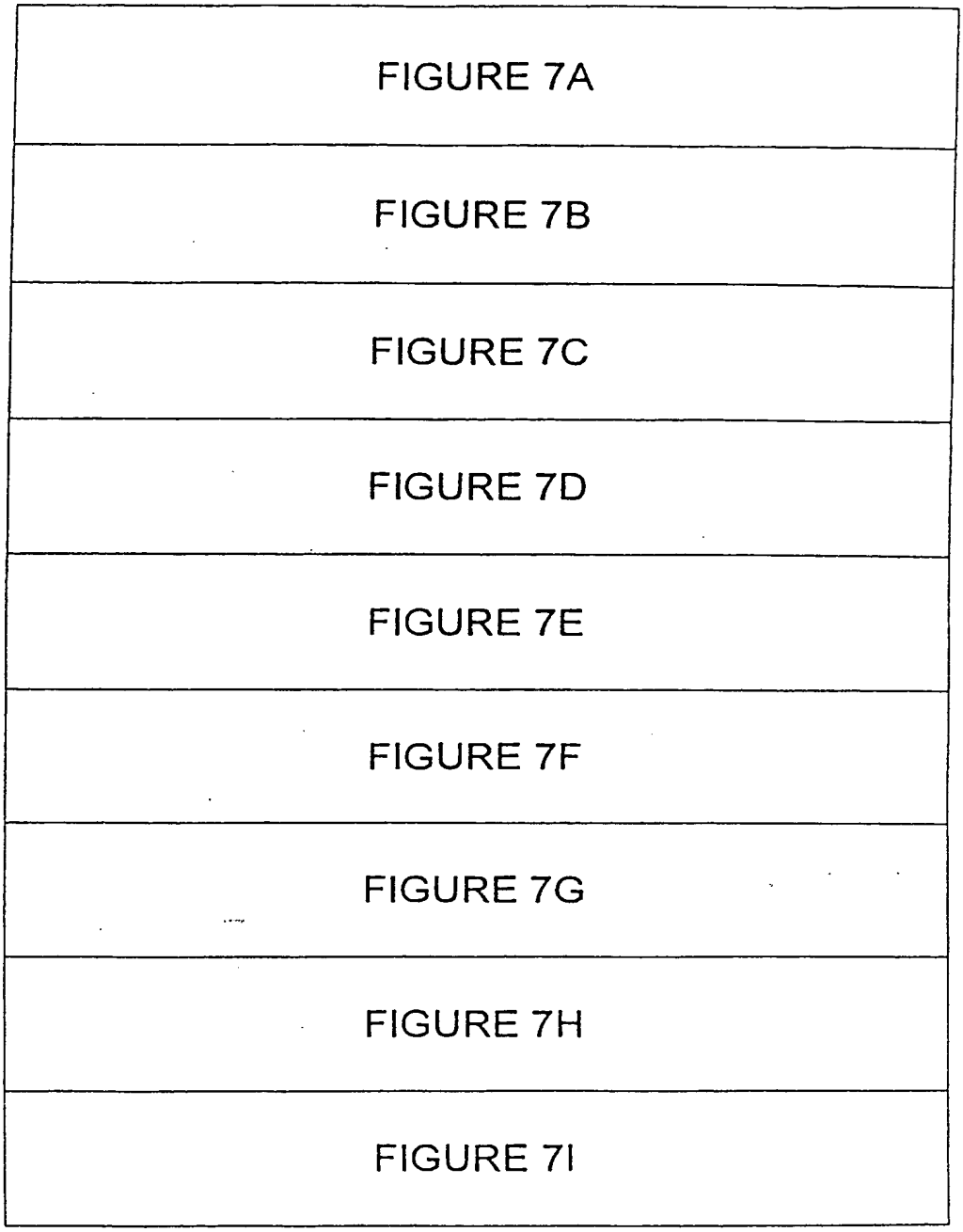


FIGURE 7A
FIGURE 7B
FIGURE 7C
FIGURE 7D
FIGURE 7E
FIGURE 7F
FIGURE 7G
FIGURE 7H
FIGURE 7I

FIGURE 7

```
          1                                                    50
wSSIII    MEMSLWPRSP LCPRSRQPLV VVRP..AGRG GLTQPFLMNG RFTRSRTLRC
mSSIII    MEMVLRSQSP LCLRS.GPVL IFRPTVAGGG GGTQSLLRTT RFARRRVIRC
pSSIII    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

          51                                                   100
wSSIII    MVASSDPPNR KSRRMVPPQV KVISSRGYTT RLIVEPSNEN TEHNNRD...
mSSIII    VVASPGCPNR KS.RTASPNV KVAAYSNYAP RLLVESSSKK SEHHDSSRHR
pSSIII    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

          101                                                  150
wSSIII    EETLDTYNAL LSTETAEWTD NREAE..... ..TAKADSSQ NALSSSIIGE
mSSIII    EETIDTYNGL SGSDAAELTS NRDVEIEVDL QHISEEELPG KVSINASLGE
pSSIII    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

          151                                                  200
wSSIII    VDVAD..... EDILAADLTV YSLSSVMKKE VDAADKARVK EDAFELDLPA
mSSIII    METVDEAEVE EDKFEVDTSG IVLRNVAVRE VDPKDEHNAK .DVFVVDSSG
pSSIII    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
```

FIGURE 7A

```
          201                                                        250
wSSIII    TTLRSVIVDV MDHNGTVQET LRSVIVDVMD .HNGTVQE.. TLRSVIVDVM
mSSIII    TAPDNAAVEE VVDEAEVEED MVDVDILGLD LNNATIEEID LMEEALLENF
pSSIII    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

          251                                                        300
wSSIII    D.DAADKARV EEDVFELDLS GNISSSAT.. .......... ......TVEL
mSSIII    DVDSPGNASS GRTYGGVDEL GELPSTSVDC IAINGKRRSL KPKPLPIVRF
pSSIII    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

          301                                                        350
wSSIII    DAVDEVGPVQ DKFEATSSGN VSNSATVREV DASDE...AG NDQGIFRADL
mSSIII    QEQEQIVLSI VDEEGLIASS CEEGQPVVDY DKQEENSTAF DEQKQLTDDF
pSSIII    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

          351                                                        400
wSSIII    SGNVFSSSTT VEVG..AVDE AGSIKDRFET DSSGNVSTSA PMWDAIDETV
mSSIII    PEEGISIVHF PEPNNDIVGS SKFLEQKQEL DGSYKQDRST TGLHEQDQSV
pSSIII    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
```

FIGURE 7B

```
        401                                                        450
wSSIII  ADQDTFEADL SGNASSCATY REVDDVVDET RSEEETFAMD LFASESGHEK
mSSIII  VSSHGQDKSI VG.VPQQIQY NDQSIAGSHR QDQSIAGAPE QIQSVAGYIK
pSSIII  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~MDVPF

        451                                                        500
wSSIII  HMAVDYVGEA TDEEETYQQQ YPVPSSFSMW DKAIAKTGVS LNPELRLVRV
mSSIII  PNQ.SIVGSC KQHELIIPEP KKIESIISYN EIDQSIVGSH KQDKSVVSVP
pSSIII  PLHRSLSCTS VSNAITHLKI KPILGFVSHG TTSLSVQSSS WRKDGMVTGV

        501                                                        550
wSSIII  EEQGKVNFSD KKDLSIDDLP GQNQSIIGSY KQDKSIADVA GPTQSIFGSS
mSSIII  EQIQSIVSHS KPNQSTVDSY RQAESIIGVP EKVQSITSYD KLDQSIVGSL
pSSIII  SFSICANFSG RRRRKVSTPR SQGSSPKGFV PRKPSGMSTQ RKVQKSNGDK

        551                                                        600
wSSIII  KQHRSIVAFP KQNQSIVSVT EQKQSIVGFR SQDLSAVSL. .........P
mSSIII  KQDEPIISVP EKIQSIVHYT KPNQSIVGLP KQQQSIVHIV EPKQSIDGFP
pSSIII  ESKSTSTSKE SEISNQKTVE ARVETSDDDT KGVVRDHKFL EDEDEINGST
```

FIGURE 7C

```
          801                                                       850
wSSIII    ADSVIDLYLN RDLTALANEP DVVIKGAFNG WKWRLFTERL HKSDLGGVWW
mSSIII    ADSTIDLYFN RDLSAVANEP DVLIKGAFNG WKWRFFTEKL HKSELAGDWW
pSSIII    PDEDVEIFLN RGLSTLKNES DVLIMGAFNE WRYRSFTTRL TETHLNGDWW

          851                                                       900
wSSIII    SCKLYIPKEA YRLDFVFFNG RTVYENNGNN DFCIGIEGTM NEDLFEDFLV
mSSIII    CCKLYIPKQA YRMDFVFFNG HTVYENNNNN DFVIQIESTM DENLFEDFLA
pSSIII    SCKIHVPKEA YRADFVFFNG QDVYDNNDGN DFSITVKGGM QIIDFENFLL

          901                                                       950
wSSIII    KEKQRELEKL AMEEAERRTQ TEEQRRRKEA RAADEAVRAQ AKAEIEIKKK
mSSIII    EEKQRELENL ANEEAERRRQ TDEQRRMEEE RAADKADRVQ AKVEVETKKN
pSSIII    EEKWREQEKL AKEQAERERL AEEQRRIEAE KAEIEADRAQ AKEEAAKKKK

          951                                                      1000
wSSIII    KLQSMLSLAR TCVDNLWYIE ASTDTRGDTI RLYYNRNSRP LAHSTEIWMH
mSSIII    KLCNVLGLAR APVDNLWYIE PITTGQEATV RLYYNINSRP LVHSTEIWMH
pSSIII    VLRELMVKAT KTRDITWYIE PSEFKCEDKV RLYYNKSSGP LSHAKDLWIH
```

FIGURE 7E

```
          601                                                  650
wSSIII    KQ.NVPIVGT SREGQTKQVP VVDRQDALYV NGLEAKEGDH TSEKTDEDAL
mSSIII    KQ.DLSIVGI SNEFQTKQLA TVGTHDGLLM KGVEAKE... TSQKTEGDTL
pSSIII    KSISMSPVRV SSQFVESEET GGDDKDAVKL N..KSKRSEE SGFIIDSVIR

          651                                                  700
wSSIII    HVKFNVDNVL RKHQADRTQA VEKKTWKKVD EEHLYMTEHQ KRAA..EGQM
mSSIII    QATFNVDNLS QKQEGLTKEA DEITIIEKIN DEDLVMIEEQ KSIAMNEEQT
pSSIII    EQSGSQGETN ASSKGSHAVG TKLYEILQVD VEPQQLKEN. .NAGNVEYKG

          701                                                  750
wSSIII    VVNEDELSIT EIGMGRGD.K IQHVLSEEEL SWSEDEVQLI EDDGQYEVDE
mSSIII    IVTEEDIPMA KVEIGIDKAK FLHLLSEEES SWDENEVGII EADEQYEVDE
pSSIII    PVASKLLEIT KA......SD VEHTESNEID DLDTN..SFF KSDLIEEDEP

          751                                                  800
wSSIII    TSVSVNVEQD IQGSPQDVVD PQALKVMLQE LAEKNYSMRN KLFVFPEVVK
mSSIII    TSMS..TEQD IQESPNDDLD PQALWSMLQE LAEKNYSLGN KLFTYPDVLK
pSSIII    LAAGTVETGD SSLNLRLEME ANLRRQAIER LAEENLLQGI RLFCFPEVVK
```

FIGURE 7D

```
          1001                                                  1050
wSSIII    GGYNNWTDGL SIVESFVKCN DKDGDWWYAD VIPPEKALVL DWVFADGPAG
mSSIII    GGYNNWIDGL SFAERLVHHH DKDCDWWFAD VVVPERTYVL DWVFADGPPG
pSSIII    GGYNNWKDGL SIVKKLVKSE RIDGDWWYTE VVIPDQALFL DWVFADGPPK

          1051                                                  1100
wSSIII    NARNYDNNAR QDFHAILPNN NVTEEGFWAQ EEQNIYTRLL QERREKEETM
mSSIII    SARNYDNNGG HDFHATLP.N NMTEEEYWME EEQRIYTRLQ QERREREEAI
pSSIII    HAIAYDNNHR QDFHAIVP.N HIPEELYWVE EEHQIFKTLQ EERRLREAAM

          1101                                                  1150
wSSIII    KRKAERSANI KAEMKAKTMR RFLLSQKHIV YTEPLEIRAG TTVDVLYNPS
mSSIII    KRKAERNAKM KAEMKEKTMR MFLVSQKHIV YTEPLEIHAG TTIDVLYNPS
pSSIII    RAKVEKTALL KTETKERTMK SFLLSQKHVV YTEPLDIQAG SSVTVYYNPA

          1151                                                  1200
wSSIII    NTVLNGKSEG WFRCSFNLWM HSSGALPPQK MVKSGDGPLL KATVDVPPDA
mSSIII    NTVLTGKPEV WFRCSFNRWM YPGGVLPPQK MVQAENGSHL KATVYVPRDA
pSSIII    NTVLNGKPEI WFRCSFNRWT HRLGPLPPQK MSPAENGTHV RATVKVPLDA
```

FIGURE 7F

```
          1201
wSSIII    YMMDFVFSEW EEDGIYDNRN GMDYHIPVSD SIETENYMRI IHIAVEMAPV
mSSIII    YMMDFVFSES EEGGIYDNRN GLDYHIPVFG SIAKEPPMHI VHIAVEMAPI
pSSIII    YMMDFVFSER EDGGIFDNKS GMDYHIPVFG GVAKEPPMHI VHIAVEMAPI

          1251
wSSIII    AKVGGLGDVV TSLSRAIQDL GHTVEVILPK YDCLNQSSVK DLHLYQSFSW
mSSIII    AKVGGLGDVV TSLSRAVQDL GHNVEVILPK YGCLNLSNVK NLQIHQSFSW
pSSIII    AKVGGLGDVV TSLSRAVQDL NHNVDIILPK YDCLKMNNVK DFRFHKNYFW

          1301
wSSIII    GGTEIKVWVG RVEDLTVYFL EPQNGMFGVG CVYG.RNDDR RFGFFCHSAL
mSSIII    GGSEINVWRG LVEGLCVYFL EPQNGMFGVG YVYG.RDDDR RFGFFCRSAL
pSSIII    GGTEIKVWFG KVEGLSVYFL EPQNGLFSKG CVYGCSNDGE RFGFFCHAAL

          1351
wSSIII    EFILQNEFSP HIIHCHDWSS APVAWLYKEH YSQSRMASTR VVFTIHNLEF
mSSIII    EFLLQSGSSP NIIHCHDWSS APVAWLHKEN YAKSSLANAR VVFTIHNLEF
pSSIII    EFLLQGGFSP DIIHCHDWSS APVAWLFKEQ YTHYGLSKSR IVFTIHNLEF
```

FIGURE 7G

```
          1401                                                      1450
wSSIII    GAHYIGKAMT YCDKATTVSP TYSRDVAGHG AIAPHREKFY GILNGIDPDI
mSSIII    GAHHIGKAMR YCDKATTVSN TYSKEVSGHG AIVPHLGKFY GILNGIDPDI
pSSIII    GADLIGRAMT NADKATTVSP TYSQEVSGNP VIAPHLHKFH GIVNGIDPDI

          1451                                                      1500
wSSIII    WDPYTDNFIP VPYTCENVVE GKRAAKRALQ QKFGLQQTDV PIVGIITRLT
mSSIII    WDPYNDNFIP VHYTCENVVE GKRAAKRALQ QKFGLQQIDV PVVGIVTRLT
pSSIII    WDPLNDKFIP IPYTSENVVE GKTAAKEALQ RKLGLKQADL PLVGIITRLT

          1501                                                      1550
wSSIII    AQKGIHLIKH AIHRTLESNG HVVLLGSAPD HRIQGDFCRL ADALHGVYHG
mSSIII    AQKGIHLIKH AIHRTLERNG QVVLLGSAPD SRIQADFVNL ANTLHGVNHG
pSSIII    HQKGIHLIKH AIWRTLERNG QVVLLGSAPD PRVQNNFVNL ANQLHSKYND

          1551                                                      1600
wSSIII    RVKLVLTYDE PLSHLIYAGS DFIIVPSIFE PCGLTQLVAM RYGSIPIVRK
mSSIII    QVRLSLTYDE PLSHLIYAGS DFILVPSIFE PCGLTQLVAM RYGTIPIVRK
pSSIII    RARLCLTYDE PLSHLIYAGA DFILVPSIFE PCGLTQLTAM RYGSIPVVRK
```

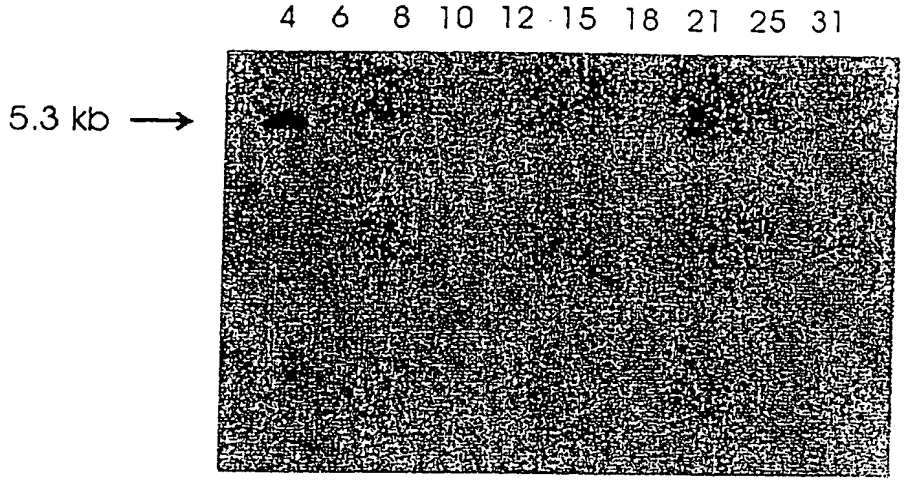
FIGURE 7H

```
                1601                                                1650
wSSIII  TGGLHDTVFD VDNDKDRARS LGLEPNGFSF DGADSNGVDY ALNRAIGAWF
mSSIII  TGGLFDTVFD VDNDKERARD RGLEPNGFSF DGADSNGVDY ALNRAISAWF
pSSIII  TGGLYDTVFD VDHDKERAQQ CGLEPNGFSF DGADAGGVDY ALNRALSAWY

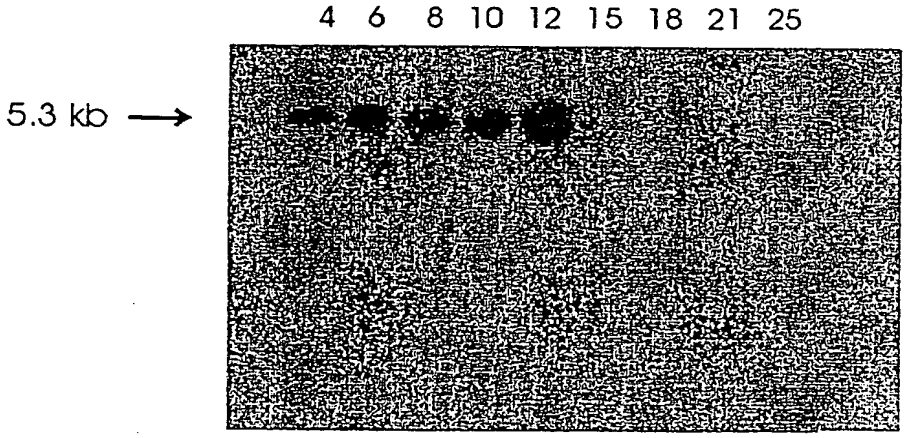
                1651                                        1689
wSSIII  DARDWFHSLC KRVMEQDWSW NRPALDYIEL YHAARKF*~
mSSIII  DARSWFHSLC KRVMEQDWSW NRPALDYIEL YRSASKL~~
pSSIII  DGRDWFNSLC KQVMEQDWSW NRPALDYLEL YHAARKLE*
```

FIGURE 7I

[a] Wyuna



[b] Gabo



[c] Gabo

FIGURE 8

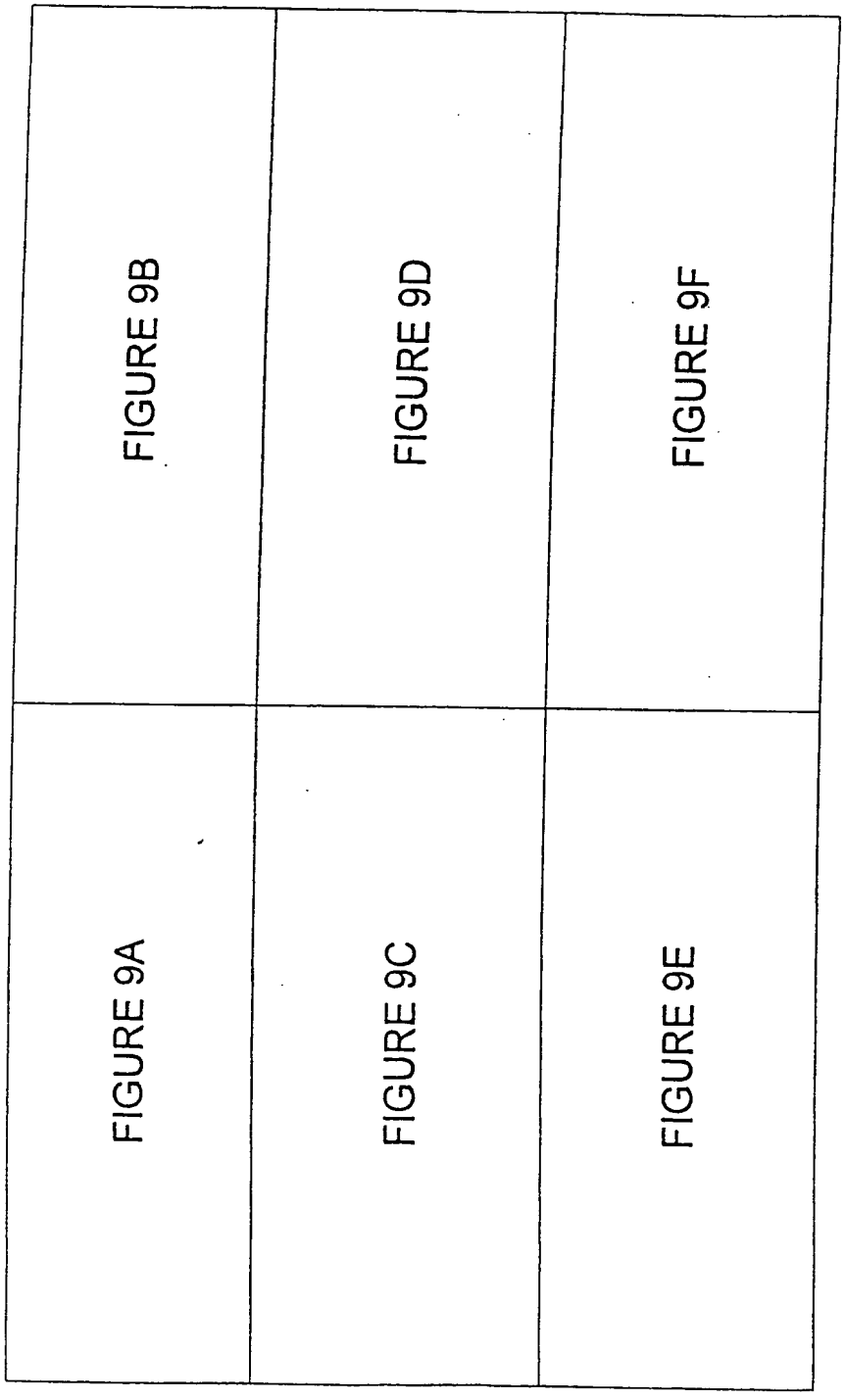


FIGURE 9

```
                                Region 1                     Region 2
                     10         20         30         40         50
wGBSS     81  FVGAEMAPWS KTGGLGDLLG GLPPAMAANG HRVMVISPRY DQYKDAWDT-
wSS1     144  -*TG*A**YA *S*****VC* S**I*L**R* *****VM*** LNGSSDKNYA
wSS2     314  --A**CS**C *******VA* A**K*L*KR* *****VV*** GD*EE*Y*V-
wSS3    1187  -IAV****VA *V*****VVT S*SR*IQDL* *T*E**L*K* *CLNQSSVK-

                    100        110        120        130        140
wGBSS    171  LEKVRGKTKE KIYGPDAGTD YEDNQQRFSL LCQAALEVPR ILNLDNNPYF
wSS1     234  -HRPGSLYGD ------NFGA FG***F*YT* **Y**C*A*L **E*GGYI*G
wSS2     404  RHRQEDIYGG ---------S RQEIMK*MI* F*K**V***W HVPCGGV**G
wSS3    1277  **PQN*MFGV ------GCVY GRNDDR**GF F*HS***--F **QNEFS*H-

                    190        200        210        220        230
wGBSS    261  FCIHNISYQG RFSFDDFAQL NLPD-----R FKSSFDFIDG YDKPVEGRKI
wSS1     324  LV***LAH** LEPASTYPD* G**PEWYGAL EWVFPEWARR HALDKGEAVN
wSS2     494  MV****AH** *GPV*E*PFT E**------- -EHYLEHFRL **PVGGEHAN
wSS3    1367  *T***L-EF* AHYIGKAMTY CDK------- ---------- ---------
```

FIGURE 9A

```
    60         70         80         90
-----SVVSE IKVVDKYERV RYFHCYKRGV DRVFVDHPCF   170
KALYTGKHIK *PCFGGSHE* TF**E*RDN* *W******SY   233
-----G*RKY Y*AAGQDME* N***A*ID** *F**I*A*L*   403
---------- -DLHLYQSFS WGGTEI*VW* G**EDLTVY*  1276

                Region 3
  150        160        170        180
SGPYGEDVVF VCNDWHTGLL ACYLKSNYQS NGIYRAAKVA   260
QN-----CM* *V****AS*V PVL*AAK*RP Y*V**DSRST   323
D*----NL** IA*****A** PV***AY*RD H*LMQYTRSI   493
--------II H*H**SSAPV *WLY*EH*SQ -SRMASTR*V  1366

  240        250        260        270
NWMKAGILQA DKVLTVSPYY AEELISGEAR GCELDNIMRL   350
FLKG*VVTAD RI*TVSQG*S W*VTTAEGGQ *LNELLSS*K   413
YFAAGLKMAD QV*VVSPG*L W*LKTVEGGW *LHDIIRQND   583
---------- --------AT TVSPTYSRDV AGHGAIAPHR  1456
```

FIGURE 9B

```
                    Region 4
                280       290       300       310       320
wGBSS   351 TGITTIVNGM DVSEWDPTKD KFLAVNYDIT TALEGKALNK EALEGKALNK
wSS1    414 SVLNG****I *IND*N**T* *C*PHH*SV- ---------- DD*S***KC*
wSS2    584 WKTRG****I *NM**N*EV* VH*KSDGYTN ------FSLG TLDS**RQC*
wSS3   1457 EKFYG*L**I *PDI***YT* N*IP*P*TCE ---NVVEG*  **AKRALQQ*

                    Region 5a
                370       380       390       400       410
wGBSS   441 LKEEDVQIVL LGTGKKKFER LLKSIEEKFP SKVRAVVRFN ------APLA
wSS1    504 *MR****F*M **S*DPI**G WMR*T*SSYK D*F*GW*G*S ------V*VS
wSS2    674 V-SQ***L*M ****RHDL*S M*RHF*REHH D***GW*G*S ------VR**
wSS3   1547 TL*SNG*V** **SAPDHRIQ GDFCRLADAL HG*YHGRVKL -VLTYDE**S
```

FIGURE 9C

```
                    Region 5
   330       340       350       360
EALQAEVGLP VDRKVPLVAF IGRLEEQKGP DVMIASIPEI  440
AE**K*L*** *RED***IG* ****DY***I *LIKMA***-  503
****R*L**Q *RAD***LG* ****DG***V EIIADAM*W*  673
FG**QT---- ---D**I*GI *T**TA***I -HL*KHAIHR 1546

   Region 6                    Region 7
   420       430       440       450
HQMMAGADVL AVTSRFEPCG LIQLQGMRYG TPCACASTGG  530
*RIT**C*I* LMP******* *N**YA*Q** *VPVVHG***  593
*RIT****A* LMP******* *N**YA*A** *VPVVHAV**  763
*LIY**S*FI I*P*I***** *T**VA**** SIPIVRK***  1636
```

FIGURE 9D

Region 7 (Continued)

```
                460         470         480         490         500

wGBSS  531 LVDTIVEGKT GFHMGRLSYD CNVVEPADVK KVVTTLKRAV KVVGTPAYIE
wSS1   594 *R**-**TFN ---------- --PFGAKGEE GTGWAFSPLT VDKMLW*LRT
wSS2   764 VR**-*PPFD ---------- --PFNHSGLG ---W*FD**E AHKLIE*LGH
wSS3  1637 ****-*FDVD NDKDRAR*LG LEPNGFSFDG ADSNGVDY*L NRAIGAWFDA

550         560         570         580         590         600
wGBSS  621 APLAMENVAA P*........ .......... .......... ..........
wSS1   684 FVDQPYVM.. .......... .......... .......... ..........
wSS2   854 KYQW...... .......... .......... .......... ..........
wSS3  1727 .......... .......... .......... .......... ..........
```

FIGURE 9E

```
                510       520       530       540
MVKNCMIQDL SWKGPAKNWE DVLLELGVEG SEPGIVGEEI  620
AMSTFREHKP **E*LM*RGM TKDHTWDHAA EQYEQIF*WA  683
CLRTYRDYKE **R*LQERGM SQDFSWEHAA KLYED*LLKA  853
RDWFHSLCKK VMEQDWSWNR PA*DYIELYH AARKF*....  1726

 610       620       630
.......... .......... .......... ..........  710
.......... .......... .......... ..........  773
.......... .......... .......... ..........  943
.......... .......... .......... ..........  1816
```

FIGURE 9F

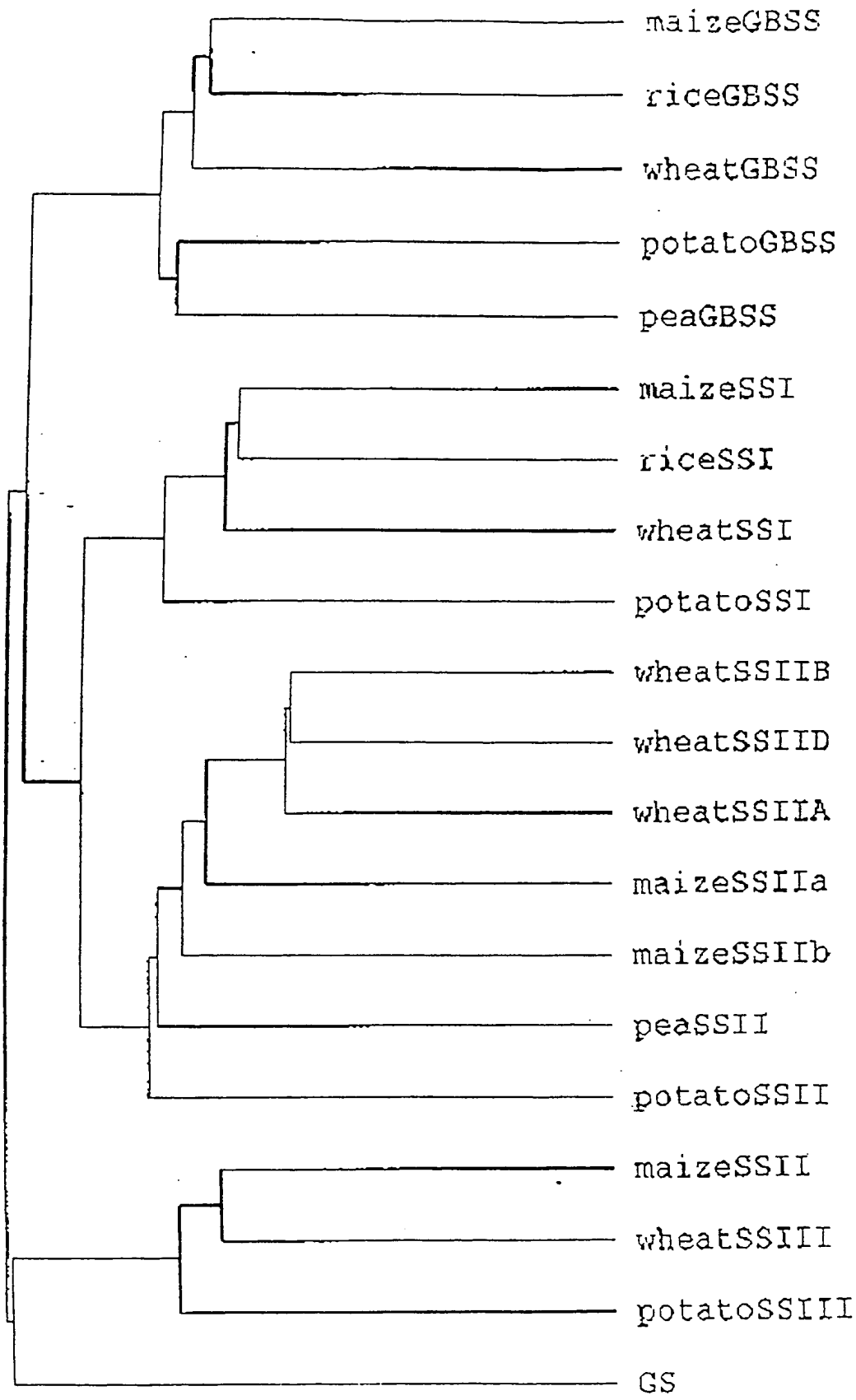

maizeGBSS
riceGBSS
wheatGBSS
potatoGBSS
peaGBSS
maizeSSI
riceSSI
wheatSSI
potatoSSI
wheatSSIIB
wheatSSIID
wheatSSIIA
maizeSSIIa
maizeSSIIb
peaSSII
potatoSSII
maizeSSII
wheatSSIII
potatoSSIII
GS

FIGURE 10

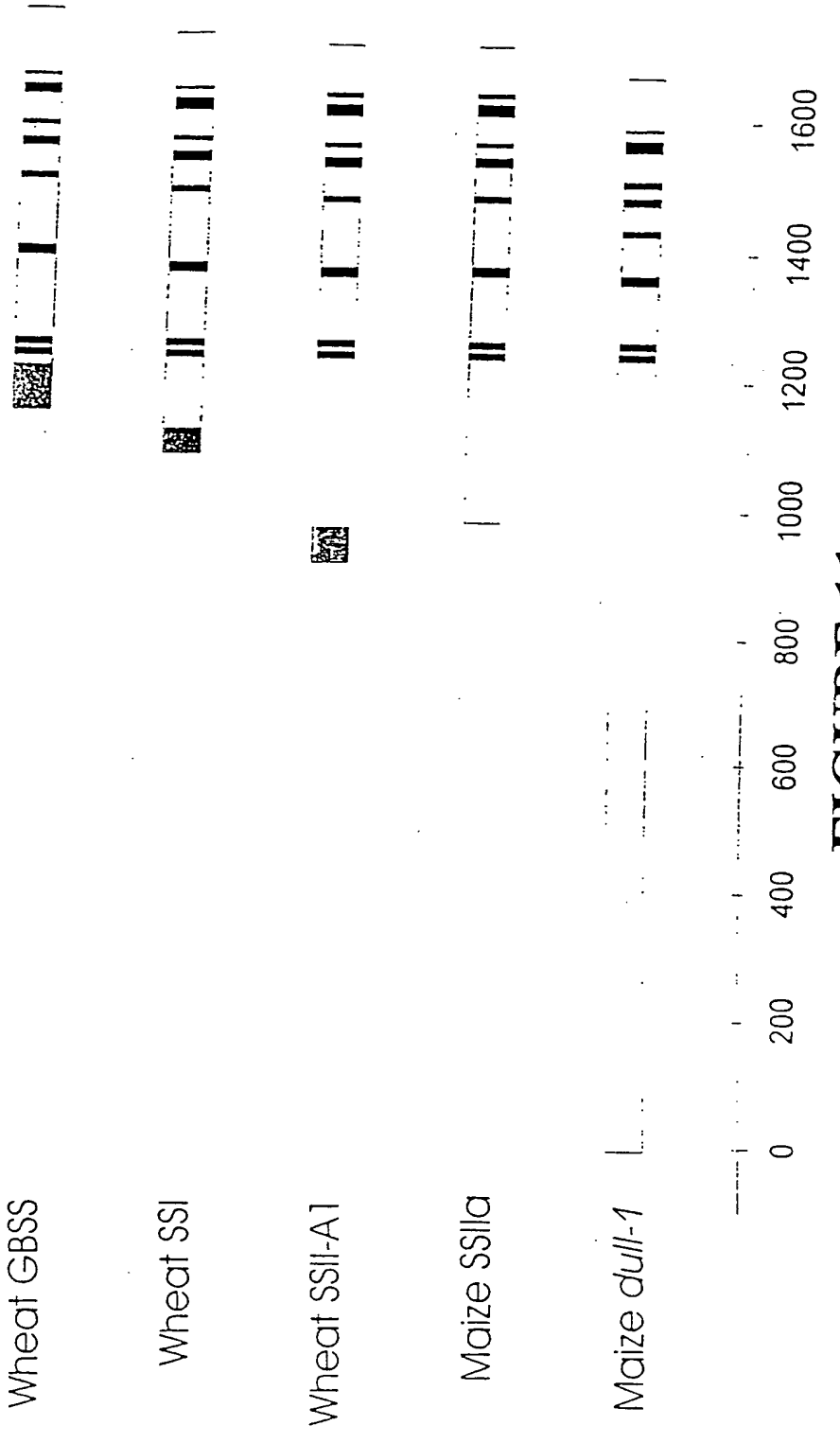

Wheat GBSS
Wheat SSI
Wheat SSII-A1
Maize SSIIa
Maize dull-1
1 2 3 4 5 5a 6 7
0
200
400
600
800
1000
1200
1400
1600

FIGURE 11

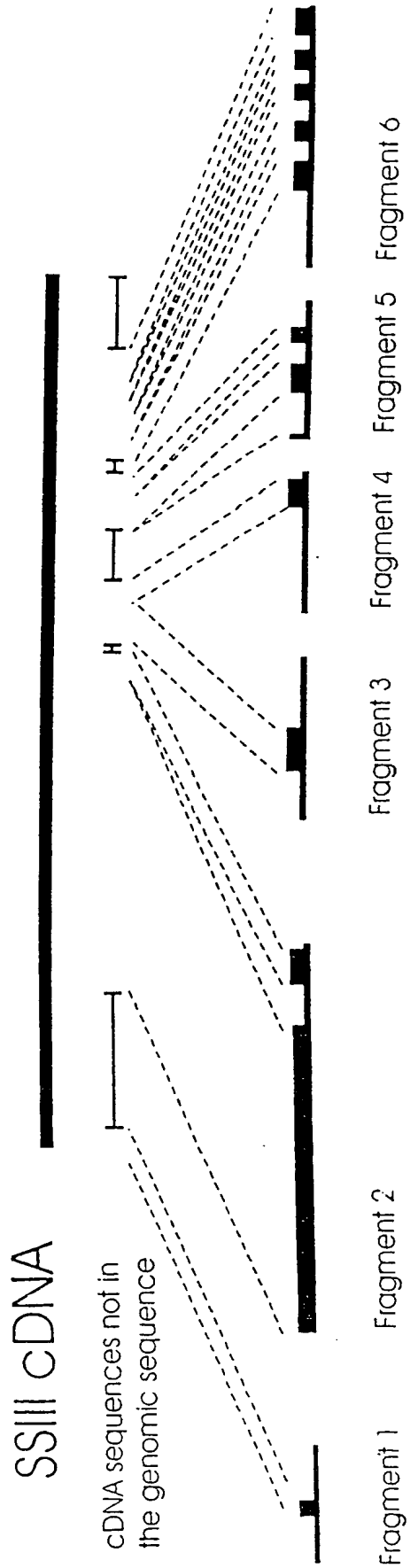

SSIII cDNA
cDNA sequences not in the genomic sequence
H
H
Fragment 1
Fragment 2
Fragment 3
Fragment 4
Fragment 5
Fragment 6

FIGURE 12

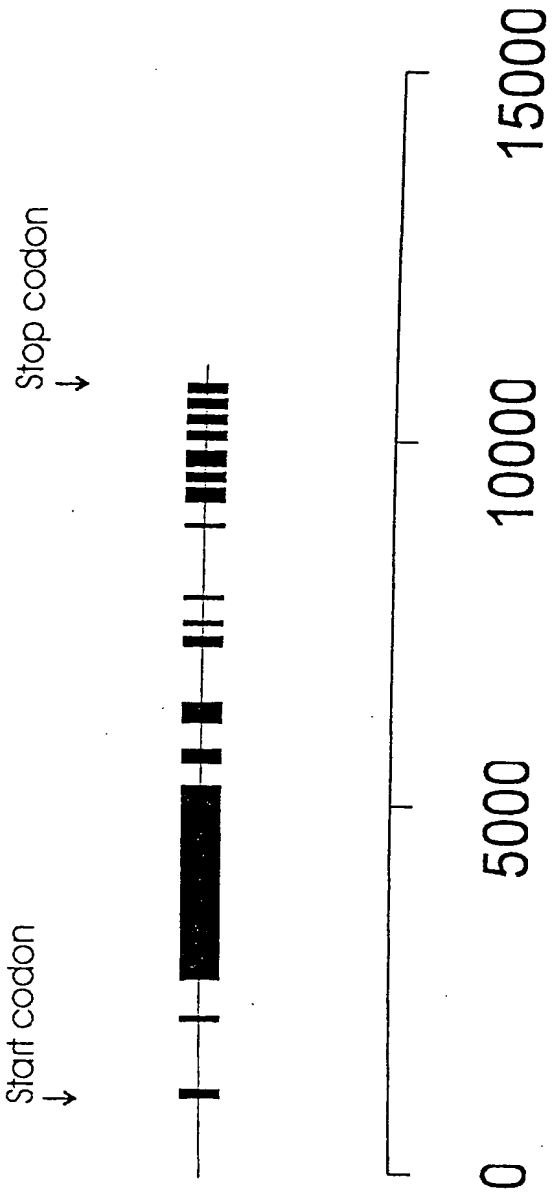


FIGURE 13

SEQUENCE LISTING

<110> COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION
GOODMAN FIELDER LIMITED
GROUPE LIMAGRAIN PACIFIC PTY LTD

<120> NOVEL GENES ENCODING WHEAT STARCH SYNTHASES AND USES THEREFOR

<130> p:\oper\mro\pi-wss.pct

<140> TO BE ADVISED
<141> 2000-04-28

<150> AU PQ0052/99
<151> 1999-04-29

<160> 54

<170> PatentIn Ver. 2.0

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<221> CDS
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cccactgccg cgctactccc cactcccact gccaccacct ccgcctgcgc cgcgctctgg 120

gcggaccaac ccgcgcatcg tatcacgatc acccaccccg atcccggccg ccgcc atg 178
Met
1

tcg tcg gcg gtc gcg tcc gcc gcg tcc ttc ctc gcg ctc gcg tcc gcc 226
Ser Ser Ala Val Ala Ser Ala Ala Ser Phe Leu Ala Leu Ala Ser Ala
5 10 15

tcc ccc ggg aga tca cgg agg agg acg agg gtg agc gcg tcg cca ccc 274
Ser Pro Gly Arg Ser Arg Arg Arg Thr Arg Val Ser Ala Ser Pro Pro
20 25 30

cac acc ggg gct ggc agg ttg cac tgg ccg ccg tcg ccg ccg cag cgc 322
His Thr Gly Ala Gly Arg Leu His Trp Pro Pro Ser Pro Pro Gln Arg
35 40 45

acg gct cgc gac gga gcg gtg gcc gcg cgc gcc gcc ggg aag aag gac 370
Thr Ala Arg Asp Gly Ala Val Ala Ala Arg Ala Ala Gly Lys Lys Asp
50 55 60 65

gcg ggg atc gac gac gcc gcg ccc gcg agg cag ccc cgc gca ctc cgc 418
Ala Gly Ile Asp Asp Ala Ala Pro Ala Arg Gln Pro Arg Ala Leu Arg
70 75 80

ggt ggc gcc gcc acc aag gtt gcg gag cgg agg gat ccc gtc aag acg 466
Gly Gly Ala Ala Thr Lys Val Ala Glu Arg Arg Asp Pro Val Lys Thr
85 90 95

ctc gat cgc gac gcc gcg gaa ggt ggc gcg ccg tcc ccg ccg gca ccg 514
Leu Asp Arg Asp Ala Ala Glu Gly Gly Ala Pro Ser Pro Pro Ala Pro

```
        100                 105                 110

agg cag gag gac gcc cgt ctg ccg agc atg aac ggc atg ccg gtg aac   562
Arg Gln Glu Asp Ala Arg Leu Pro Ser Met Asn Gly Met Pro Val Asn
    115                 120                 125

ggt gaa aac aaa tct acc ggc ggc ggc ggc gcg act aaa gac agc ggg   610
Gly Glu Asn Lys Ser Thr Gly Gly Gly Gly Ala Thr Lys Asp Ser Gly
130                 135                 140                 145

ctg ccc gca ccc gca cgc gcg ccc cag ccg tcg agc cag aac aga gta   658
Leu Pro Ala Pro Ala Arg Ala Pro Gln Pro Ser Ser Gln Asn Arg Val
                150                 155                 160

ccg gtg aat ggt gaa aac aaa gct aac gtc gcc tcg ccg ccg acg agc   706
Pro Val Asn Gly Glu Asn Lys Ala Asn Val Ala Ser Pro Pro Thr Ser
            165                 170                 175

ata gcc gag gtc gcg gct ccg gat ccc gca gct acc att tcc atc agt   754
Ile Ala Glu Val Ala Ala Pro Asp Pro Ala Ala Thr Ile Ser Ile Ser
        180                 185                 190

gac aag gcg cca gag tcc gtt gtc cca gcc gag aag gcg ccg ccg tcg   802
Asp Lys Ala Pro Glu Ser Val Val Pro Ala Glu Lys Ala Pro Pro Ser
    195                 200                 205

tcc ggc tca aat ttc gtg ccc tcg gct tct gct ccc ggg tct gac act   850
Ser Gly Ser Asn Phe Val Pro Ser Ala Ser Ala Pro Gly Ser Asp Thr
210                 215                 220                 225

gtc agc gac gtg gaa ctt gaa ctg aag aag ggt gcg gtc att gtc aaa   898
Val Ser Asp Val Glu Leu Glu Leu Lys Lys Gly Ala Val Ile Val Lys
                230                 235                 240

gaa gct cca aac cca aag gct ctt tcg ccg ccc gca gca ccc gct gta   946
Glu Ala Pro Asn Pro Lys Ala Leu Ser Pro Pro Ala Ala Pro Ala Val
            245                 250                 255

caa caa gac ctt tgg gac ttc aag aaa tac att ggt ttc gag gag ccc   994
Gln Gln Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe Glu Glu Pro
        260                 265                 270

gtg gag gcc aag gat gat ggc cgg gct gtt gca gat gat gcg ggc tcc   1042
Val Glu Ala Lys Asp Asp Gly Arg Ala Val Ala Asp Asp Ala Gly Ser
    275                 280                 285

ttc gaa cac cac cag aat cac gat tcc ggg cct ttg gca ggg gag aac   1090
Phe Glu His His Gln Asn His Asp Ser Gly Pro Leu Ala Gly Glu Asn
290                 295                 300                 305

gtc atg aac gtg gtc gtc gtg gct gct gaa tgt tct ccc tgg tgc aaa   1138
Val Met Asn Val Val Val Val Ala Ala Glu Cys Ser Pro Trp Cys Lys
                310                 315                 320

aca ggt ggt ctt gga gat gtt gcc ggt gct ttg ccc aag gct ttg gcg   1186
Thr Gly Gly Leu Gly Asp Val Ala Gly Ala Leu Pro Lys Ala Leu Ala
            325                 330                 335

aag aga gga cat cgt gtt atg gtt gtg gta cca agg tat ggg gac tat   1234
Lys Arg Gly His Arg Val Met Val Val Val Pro Arg Tyr Gly Asp Tyr
        340                 345                 350

gag gaa gcc tac gat gtc gga gtc cga aaa tac tac aag gct gct gga   1282
Glu Glu Ala Tyr Asp Val Gly Val Arg Lys Tyr Tyr Lys Ala Ala Gly
    355                 360                 365
```

```
cag gat atg gaa gtg aat tat ttc cat gct tat atc gat gga gtt gat   1330
Gln Asp Met Glu Val Asn Tyr Phe His Ala Tyr Ile Asp Gly Val Asp
370                 375                 380                 385

ttt gtg ttc att gac gct cct ctc ttc cga cac cgc cag gaa gac att   1378
Phe Val Phe Ile Asp Ala Pro Leu Phe Arg His Arg Gln Glu Asp Ile
                390                 395                 400

tat ggg ggc agc aga cag gaa att atg aag cgc atg att ttg ttc tgc   1426
Tyr Gly Gly Ser Arg Gln Glu Ile Met Lys Arg Met Ile Leu Phe Cys
            405                 410                 415

aag gcc gct gtc gag gtt cca tgg cac gtt cca tgc ggc ggt gtc cct   1474
Lys Ala Ala Val Glu Val Pro Trp His Val Pro Cys Gly Gly Val Pro
        420                 425                 430

tat ggg gat gga aat ctg gtg ttt att gca aat gat tgg cac acg gca   1522
Tyr Gly Asp Gly Asn Leu Val Phe Ile Ala Asn Asp Trp His Thr Ala
    435                 440                 445

ctc ctg cct gtc tat ctg aaa gca tat tac agg gac cat ggt ttg atg   1570
Leu Leu Pro Val Tyr Leu Lys Ala Tyr Tyr Arg Asp His Gly Leu Met
450                 455                 460                 465

cag tac act cgg tcc att atg gtg ata cat aac atc gct cac cag ggc   1618
Gln Tyr Thr Arg Ser Ile Met Val Ile His Asn Ile Ala His Gln Gly
                470                 475                 480

cgt ggc cca gta gat gag ttc ccg ttc acc gag ttg cct gag cac tac   1666
Arg Gly Pro Val Asp Glu Phe Pro Phe Thr Glu Leu Pro Glu His Tyr
            485                 490                 495

ctg gaa cac ttc aga ctg tac gac ccc gtg ggt ggt gaa cac gcc aac   1714
Leu Glu His Phe Arg Leu Tyr Asp Pro Val Gly Gly Glu His Ala Asn
        500                 505                 510

tac ttc gcc gcc ggc ctg aag atg gcg gac cag gtt gtc gtc gtg agc   1762
Tyr Phe Ala Ala Gly Leu Lys Met Ala Asp Gln Val Val Val Val Ser
    515                 520                 525

ccg ggg tac ctg tgg gag ctg aag acg gtg gag ggc ggc tgg ggg ctt   1810
Pro Gly Tyr Leu Trp Glu Leu Lys Thr Val Glu Gly Gly Trp Gly Leu
530                 535                 540                 545

cac gac atc ata cgg cag aac gac tgg aag acc cgc ggc atc gtg aac   1858
His Asp Ile Ile Arg Gln Asn Asp Trp Lys Thr Arg Gly Ile Val Asn
                550                 555                 560

ggc atc gac aac atg gag tgg aac ccc gag gtg gac gtc cac ctc aag   1906
Gly Ile Asp Asn Met Glu Trp Asn Pro Glu Val Asp Val His Leu Lys
            565                 570                 575

tcg gac ggc tac acc aac ttc tcc ctg ggg acg ctg gac tcc ggc aag   1954
Ser Asp Gly Tyr Thr Asn Phe Ser Leu Gly Thr Leu Asp Ser Gly Lys
        580                 585                 590

cgg cag tgc aag gag gcc ctg cag cgg gag ctg ggc ctg cag gtc cgc   2002
Arg Gln Cys Lys Glu Ala Leu Gln Arg Glu Leu Gly Leu Gln Val Arg
    595                 600                 605

ggc gac gtg ccg ctg ctc ggc ttc atc ggg cgc ctg gac ggg cag aag   2050
Gly Asp Val Pro Leu Leu Gly Phe Ile Gly Arg Leu Asp Gly Gln Lys
610                 615                 620                 625
```

ggc gtg gag atc atc gcg gac gcg atg ccc tgg atc gtg agc cag gac 2098
Gly Val Glu Ile Ile Ala Asp Ala Met Pro Trp Ile Val Ser Gln Asp
630 635 640

gtg cag ctg gtc atg ctg ggc acc ggg cgc cac gac ctg gag ggc atg 2146
Val Gln Leu Val Met Leu Gly Thr Gly Arg His Asp Leu Glu Gly Met
645 650 655

ctg cgg cac ttc gag cgg gag cac cac gac aag gtg cgc ggg tgg gtg 2194
Leu Arg His Phe Glu Arg Glu His His Asp Lys Val Arg Gly Trp Val
660 665 670

ggg ttc tcc gtg cgg ctg gcg cac cgg atc acg gcc ggc gcc gac gcg 2242
Gly Phe Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly Ala Asp Ala
675 680 685

ctc ctc atg ccc tcc cgg ttc gag ccg tgc gga ctg aac cag ctc tac 2290
Leu Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr
690 695 700 705

gcc atg gcc tac ggc acc gtc ccc gtc gtg cat gcc gtc ggt ggc ctg 2338
Ala Met Ala Tyr Gly Thr Val Pro Val Val His Ala Val Gly Gly Leu
710 715 720

agg gac acc gtg ccg ccg ttc gac ccc ttc aac cac tcc ggg ctc ggg 2386
Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly Leu Gly
725 730 735

tgg acg ttc gac cgc gca gag gcg cag aag ctg atc gag gcg ctc ggg 2434
Trp Thr Phe Asp Arg Ala Glu Ala Gln Lys Leu Ile Glu Ala Leu Gly
740 745 750

cac tgc ctc cgc acc tac cgg gac tac aag gag agc tgg agg ggg ctc 2482
His Cys Leu Arg Thr Tyr Arg Asp Tyr Lys Glu Ser Trp Arg Gly Leu
755 760 765

cag gag cgc ggc atg tcg cag gac ttc agc tgg gag cat gcc gcc aag 2530
Gln Glu Arg Gly Met Ser Gln Asp Phe Ser Trp Glu His Ala Ala Lys
770 775 780 785

ctc tac gag gac gtc ctc gtc aag gcc aag tac cag tgg tgaacgctag 2579
Leu Tyr Glu Asp Val Leu Val Lys Ala Lys Tyr Gln Trp
790 795

ctgctagccg gtccagcccc gcatgcgtgc atgacaggat ggaattgcgc attgcgcacg 2639

caggaaggtg ccatggagcg ccggcatccg cgaagtacag tgacatgagg tgtgtgtggt 2699

tgagacgctg attccgatct ggtccgtagc agagtagagc ggaggtaggg aagcgctcct 2759

tgttacaggt atatgggaat gttgttaact tggtattgta atttgttatg ttgtgtgcat 2819

tattacagag ggcaacgatc tgcgccggcg caccggccca actgttgggc cggtcgcaca 2879

gcagccgttg gatccgaccg cctgggccgt tggatcccac cgaaaaaaaa aaaaaaaaaa 2939

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<211> 798
<212> PRT
<213> Triticum aestivum

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1 5 10 15

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Ala Ser Pro Gly Arg Ser Arg Arg Arg Thr Arg Val Ser Ala Ser Pro
            20                  25                  30

Pro His Thr Gly Ala Gly Arg Leu His Trp Pro Pro Ser Pro Pro Gln
        35                  40                  45

Arg Thr Ala Arg Asp Gly Ala Val Ala Ala Arg Ala Ala Gly Lys Lys
    50                  55                  60

Asp Ala Gly Ile Asp Asp Ala Ala Pro Ala Arg Gln Pro Arg Ala Leu
65                  70                  75                  80

Arg Gly Gly Ala Ala Thr Lys Val Ala Glu Arg Arg Asp Pro Val Lys
                85                  90                  95

Thr Leu Asp Arg Asp Ala Ala Glu Gly Gly Ala Pro Ser Pro Pro Ala
            100                 105                 110

Pro Arg Gln Glu Asp Ala Arg Leu Pro Ser Met Asn Gly Met Pro Val
        115                 120                 125

Asn Gly Glu Asn Lys Ser Thr Gly Gly Gly Gly Ala Thr Lys Asp Ser
    130                 135                 140

Gly Leu Pro Ala Pro Ala Arg Ala Pro Gln Pro Ser Ser Gln Asn Arg
145                 150                 155                 160

Val Pro Val Asn Gly Glu Asn Lys Ala Asn Val Ala Ser Pro Pro Thr
                165                 170                 175

Ser Ile Ala Glu Val Ala Ala Pro Asp Pro Ala Ala Thr Ile Ser Ile
            180                 185                 190

Ser Asp Lys Ala Pro Glu Ser Val Val Pro Ala Glu Lys Ala Pro Pro
        195                 200                 205

Ser Ser Gly Ser Asn Phe Val Pro Ser Ala Ser Ala Pro Gly Ser Asp
    210                 215                 220

Thr Val Ser Asp Val Glu Leu Glu Leu Lys Lys Gly Ala Val Ile Val
225                 230                 235                 240

Lys Glu Ala Pro Asn Pro Lys Ala Leu Ser Pro Pro Ala Ala Pro Ala
                245                 250                 255

Val Gln Gln Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe Glu Glu
            260                 265                 270

Pro Val Glu Ala Lys Asp Asp Gly Arg Ala Val Ala Asp Asp Ala Gly
        275                 280                 285

Ser Phe Glu His His Gln Asn His Asp Ser Gly Pro Leu Ala Gly Glu
    290                 295                 300

Asn Val Met Asn Val Val Val Val Ala Ala Glu Cys Ser Pro Trp Cys
305                 310                 315                 320

Lys Thr Gly Gly Leu Gly Asp Val Ala Gly Ala Leu Pro Lys Ala Leu
                325                 330                 335

Ala Lys Arg Gly His Arg Val Met Val Val Val Pro Arg Tyr Gly Asp
            340                 345                 350

Tyr Glu Glu Ala Tyr Asp Val Gly Val Arg Lys Tyr Tyr Lys Ala Ala
```

```
        355                 360                 365

Gly Gln Asp Met Glu Val Asn Tyr Phe His Ala Tyr Ile Asp Gly Val
    370                 375                 380

Asp Phe Val Phe Ile Asp Ala Pro Leu Phe Arg His Arg Gln Glu Asp
385                 390                 395                 400

Ile Tyr Gly Gly Ser Arg Gln Glu Ile Met Lys Arg Met Ile Leu Phe
                405                 410                 415

Cys Lys Ala Ala Val Glu Val Pro Trp His Val Pro Cys Gly Gly Val
            420                 425                 430

Pro Tyr Gly Asp Gly Asn Leu Val Phe Ile Ala Asn Asp Trp His Thr
        435                 440                 445

Ala Leu Leu Pro Val Tyr Leu Lys Ala Tyr Tyr Arg Asp His Gly Leu
    450                 455                 460

Met Gln Tyr Thr Arg Ser Ile Met Val Ile His Asn Ile Ala His Gln
465                 470                 475                 480

Gly Arg Gly Pro Val Asp Glu Phe Pro Phe Thr Glu Leu Pro Glu His
                485                 490                 495

Tyr Leu Glu His Phe Arg Leu Tyr Asp Pro Val Gly Gly Glu His Ala
            500                 505                 510

Asn Tyr Phe Ala Ala Gly Leu Lys Met Ala Asp Gln Val Val Val Val
        515                 520                 525

Ser Pro Gly Tyr Leu Trp Glu Leu Lys Thr Val Glu Gly Gly Trp Gly
    530                 535                 540

Leu His Asp Ile Ile Arg Gln Asn Asp Trp Lys Thr Arg Gly Ile Val
545                 550                 555                 560

Asn Gly Ile Asp Asn Met Glu Trp Asn Pro Glu Val Asp Val His Leu
                565                 570                 575

Lys Ser Asp Gly Tyr Thr Asn Phe Ser Leu Gly Thr Leu Asp Ser Gly
            580                 585                 590

Lys Arg Gln Cys Lys Glu Ala Leu Gln Arg Glu Leu Gly Leu Gln Val
        595                 600                 605

Arg Gly Asp Val Pro Leu Leu Gly Phe Ile Gly Arg Leu Asp Gly Gln
    610                 615                 620

Lys Gly Val Glu Ile Ile Ala Asp Ala Met Pro Trp Ile Val Ser Gln
625                 630                 635                 640

Asp Val Gln Leu Val Met Leu Gly Thr Gly Arg His Asp Leu Glu Gly
                645                 650                 655

Met Leu Arg His Phe Glu Arg Glu His His Asp Lys Val Arg Gly Trp
            660                 665                 670

Val Gly Phe Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly Ala Asp
        675                 680                 685

Ala Leu Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln Leu
    690                 695                 700
```

```
Tyr Ala Met Ala Tyr Gly Thr Val Pro Val Val His Ala Val Gly Gly
705                 710                 715                 720

Leu Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly Leu
                725                 730                 735

Gly Trp Thr Phe Asp Arg Ala Glu Ala Gln Lys Leu Ile Glu Ala Leu
            740                 745                 750

Gly His Cys Leu Arg Thr Tyr Arg Asp Tyr Lys Glu Ser Trp Arg Gly
        755                 760                 765

Leu Gln Glu Arg Gly Met Ser Gln Asp Phe Ser Trp Glu His Ala Ala
    770                 775                 780

Lys Leu Tyr Glu Asp Val Leu Val Lys Ala Lys Tyr Gln Trp
785                 790                 795


<210> 3
<211> 2842
<212> DNA
<213> Triticum aestivum

<220>
<221> CDS
<222> (89)..(2485)

<400> 3
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atcgcccgcc ccgatcccgg ccgccgcc atg tcg tcg gcg gtc gcg tcc gcc    112
                                Met Ser Ser Ala Val Ala Ser Ala
                                  1               5

gcg tcc ttc ctc gcg ctc gcc tcc gcc tcc ccc ggg aga tca cgc agg   160
Ala Ser Phe Leu Ala Leu Ala Ser Ala Ser Pro Gly Arg Ser Arg Arg
     10                  15                  20

cgg gcg agg gtg agc gcg ccg cca ccc cac gcc ggg gcc ggc agg ctg   208
Arg Ala Arg Val Ser Ala Pro Pro Pro His Ala Gly Ala Gly Arg Leu
 25                  30                  35                  40

cac tgg ccg ccg tgg ccg ccg cag cgc acg gct cgc gac gga ggt gtg   256
His Trp Pro Pro Trp Pro Pro Gln Arg Thr Ala Arg Asp Gly Gly Val
                 45                  50                  55

gcc gcg cgc gcc gcc ggg aag aag gac gcg agg gtc gac gac gac gcc   304
Ala Ala Arg Ala Ala Gly Lys Lys Asp Ala Arg Val Asp Asp Asp Ala
            60                  65                  70

gcg tcc gcg agg cag ccc cgc gca cgc cgc ggt ggc gcc gcc acc aag   352
Ala Ser Ala Arg Gln Pro Arg Ala Arg Arg Gly Gly Ala Ala Thr Lys
        75                  80                  85

gtc gcg gag cgg agg gat ccc gtc aag acg ctc gat cgc gac gcc gcg   400
Val Ala Glu Arg Arg Asp Pro Val Lys Thr Leu Asp Arg Asp Ala Ala
     90                  95                  100

gaa ggt ggc gcg ccg gca ccg ccg gca ccg agg cag gac gcc gcc cgt   448
Glu Gly Gly Ala Pro Ala Pro Pro Ala Pro Arg Gln Asp Ala Ala Arg
105                 110                 115                 120

cca ccg agt atg aac ggc acg ccg gtg aac ggt gag aac aaa tct acc   496
Pro Pro Ser Met Asn Gly Thr Pro Val Asn Gly Glu Asn Lys Ser Thr
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                125                 130                 135

ggc ggc ggc ggc gcg acc aaa gac agc ggg ctg ccc gca ccc gca cgc   544
Gly Gly Gly Gly Ala Thr Lys Asp Ser Gly Leu Pro Ala Pro Ala Arg
            140                 145                 150

gcg ccc cat ccg tcg acc cag aac aga gta cca gtg aac ggt gaa aac   592
Ala Pro His Pro Ser Thr Gln Asn Arg Val Pro Val Asn Gly Glu Asn
        155                 160                 165

aaa gct aac gtc gcc tcg ccg ccg acg agc ata gcc gag gtc gtg gct   640
Lys Ala Asn Val Ala Ser Pro Pro Thr Ser Ile Ala Glu Val Val Ala
    170                 175                 180

ccg gat tcc gca gct acc att tcc atc agt gac aag gcg ccg gag tcc   688
Pro Asp Ser Ala Ala Thr Ile Ser Ile Ser Asp Lys Ala Pro Glu Ser
185                 190                 195                 200

gtt gtc cca gcc gag aag ccg ccg ccg tcg tcc ggc tca aat ttc gtg   736
Val Val Pro Ala Glu Lys Pro Pro Pro Ser Ser Gly Ser Asn Phe Val
                205                 210                 215

gtc tcg gct tct gct ccc agg ctg gac att gac agc gat gtt gaa cct   784
Val Ser Ala Ser Ala Pro Arg Leu Asp Ile Asp Ser Asp Val Glu Pro
            220                 225                 230

gaa ctg aag aag ggt gcg gtc atc gtc gaa gaa gct cca aac cca aag   832
Glu Leu Lys Lys Gly Ala Val Ile Val Glu Glu Ala Pro Asn Pro Lys
        235                 240                 245

gct ctt tcg ccg cct gca gcc ccc gct gta caa gaa gac ctt tgg gac   880
Ala Leu Ser Pro Pro Ala Ala Pro Ala Val Gln Glu Asp Leu Trp Asp
    250                 255                 260

ttc aag aaa tac att ggc ttc gag gag ccc gtg gag gcc aag gat gat   928
Phe Lys Lys Tyr Ile Gly Phe Glu Glu Pro Val Glu Ala Lys Asp Asp
265                 270                 275                 280

ggc tgg gct gtt gca gat gat gcg ggc tcc ttt gaa cat cac cag aac   976
Gly Trp Ala Val Ala Asp Asp Ala Gly Ser Phe Glu His His Gln Asn
                285                 290                 295

cat gat tcc gga cct ttg gca ggg gag aac gtc atg aac gtg gtc gtc   1024
His Asp Ser Gly Pro Leu Ala Gly Glu Asn Val Met Asn Val Val Val
            300                 305                 310

gtg gct gct gaa tgt tct ccc tgg tgc aaa aca ggt ggt ctt gga gat   1072
Val Ala Ala Glu Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp
        315                 320                 325

gtt gcc ggt gct ttg ccc aag gct ttg gcg aag aga gga cat cgt gtt   1120
Val Ala Gly Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His Arg Val
    330                 335                 340

atg gtt gtg gta cca agg tat ggg gac tat gag gaa gcc tac gat gtc   1168
Met Val Val Val Pro Arg Tyr Gly Asp Tyr Glu Glu Ala Tyr Asp Val
345                 350                 355                 360

gga gtc cga aaa tac tac aag gct gct gga cag gat atg gaa gtg aat   1216
Gly Val Arg Lys Tyr Tyr Lys Ala Ala Gly Gln Asp Met Glu Val Asn
                365                 370                 375

tat ttc cat gct tat atc gat gga gtt gat ttt gtg ttc att gac gct   1264
Tyr Phe His Ala Tyr Ile Asp Gly Val Asp Phe Val Phe Ile Asp Ala
            380                 385                 390
```

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cct ctc ttc cga cac cgc cag gaa gac att tat ggg ggc agc aga cag   1312
Pro Leu Phe Arg His Arg Gln Glu Asp Ile Tyr Gly Gly Ser Arg Gln
        395                 400                 405

gaa att atg aag cgc atg att ttg ttc tgc aag gcc gct gtc gag gtt   1360
Glu Ile Met Lys Arg Met Ile Leu Phe Cys Lys Ala Ala Val Glu Val
    410                 415                 420

cct tgg cac gtt cca tgc ggc ggt gtc cct tat ggg gat gga aat ctg   1408
Pro Trp His Val Pro Cys Gly Gly Val Pro Tyr Gly Asp Gly Asn Leu
425                 430                 435                 440

gtg ttt att gca aat gat tgg cac acg gca ctc ctg cct gtc tat ctg   1456
Val Phe Ile Ala Asn Asp Trp His Thr Ala Leu Leu Pro Val Tyr Leu
                445                 450                 455

aaa gca tat tac agg gac cat ggt ttg atg cag tac act cgg tcc att   1504
Lys Ala Tyr Tyr Arg Asp His Gly Leu Met Gln Tyr Thr Arg Ser Ile
            460                 465                 470

atg gtg ata cat aac atc gcg cac cag ggc cgt ggc cca gta gat gaa   1552
Met Val Ile His Asn Ile Ala His Gln Gly Arg Gly Pro Val Asp Glu
        475                 480                 485

ttc ccg ttc acc gag ttg cct gag cac tac ctg gaa cac ttc aga ctg   1600
Phe Pro Phe Thr Glu Leu Pro Glu His Tyr Leu Glu His Phe Arg Leu
    490                 495                 500

tac gac ccc gtg ggt ggt gag cac gcc aac tac ttc gcc gcc ggc ctg   1648
Tyr Asp Pro Val Gly Gly Glu His Ala Asn Tyr Phe Ala Ala Gly Leu
505                 510                 515                 520

aag atg gcg gac cag gtt gtc gtg gtg agc ccc ggg tac ctg tgg gag   1696
Lys Met Ala Asp Gln Val Val Val Val Ser Pro Gly Tyr Leu Trp Glu
                525                 530                 535

ctc aag acg gtg gag ggc ggc tgg ggg ctt cac gac atc ata cgg cag   1744
Leu Lys Thr Val Glu Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln
            540                 545                 550

aac gac tgg aag acc cgc ggc atc gtc aac ggc atc gac aac atg gag   1792
Asn Asp Trp Lys Thr Arg Gly Ile Val Asn Gly Ile Asp Asn Met Glu
        555                 560                 565

tgg aac ccc gag gtg gac gtc cac ctc aag tcg gac ggc tac acc aac   1840
Trp Asn Pro Glu Val Asp Val His Leu Lys Ser Asp Gly Tyr Thr Asn
    570                 575                 580

ttc tcc ctg ggg acg ctg gac tcc ggc aag cgg cag tgc aag gag gcc   1888
Phe Ser Leu Gly Thr Leu Asp Ser Gly Lys Arg Gln Cys Lys Glu Ala
585                 590                 595                 600

ctg cag cgc gag ctg ggc ctg cag gtc cgc gcc gac gtg ccg ctg ctc   1936
Leu Gln Arg Glu Leu Gly Leu Gln Val Arg Ala Asp Val Pro Leu Leu
                605                 610                 615

ggc ttc atc ggc cgc ctg gac ggg cag aag ggc gtg gag atc atc gcg   1984
Gly Phe Ile Gly Arg Leu Asp Gly Gln Lys Gly Val Glu Ile Ile Ala
            620                 625                 630

gac gcc atg ccc tgg atc gtg agc cag gac gtg cag ctg gtc atg ctg   2032
Asp Ala Met Pro Trp Ile Val Ser Gln Asp Val Gln Leu Val Met Leu
        635                 640                 645
```

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ggc acc ggc cgc cac gac ctg gag agc atg ctg cgg cac ttc gag cgg   2080
Gly Thr Gly Arg His Asp Leu Glu Ser Met Leu Arg His Phe Glu Arg
    650                 655                 660

gag cac cac gac aag gtg cgc ggg tgg gtg ggg ttc tcc gtg cgc ctg   2128
Glu His His Asp Lys Val Arg Gly Trp Val Gly Phe Ser Val Arg Leu
665                 670                 675                 680

gcg cac cgg atc acg gcg ggc gcc gac gcg ctc ctc atg ccc tcc cgg   2176
Ala His Arg Ile Thr Ala Gly Ala Asp Ala Leu Leu Met Pro Ser Arg
                685                 690                 695

ttc gag ccg tgc ggg ttg aac cag ctt tac gcc atg gcc tac ggc acc   2224
Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr
            700                 705                 710

gtc ccc gtc gtg cac gcc gtc ggc ggg gtg agg gac acc gtg ccg ccg   2272
Val Pro Val Val His Ala Val Gly Gly Val Arg Asp Thr Val Pro Pro
        715                 720                 725

ttc gac ccc ttc aac cac tcc ggc ctc ggg tgg acg ttc gac cgc gcc   2320
Phe Asp Pro Phe Asn His Ser Gly Leu Gly Trp Thr Phe Asp Arg Ala
    730                 735                 740

gag gcg cac aag ctg atc gag gcg ctc ggg cac tgc ctc cgc acc tac   2368
Glu Ala His Lys Leu Ile Glu Ala Leu Gly His Cys Leu Arg Thr Tyr
745                 750                 755                 760

cgg gac tac aag gag agc tgg agg ggc ctc cag gag cgc ggc atg tcg   2416
Arg Asp Tyr Lys Glu Ser Trp Arg Gly Leu Gln Glu Arg Gly Met Ser
                765                 770                 775

cag gac ttc agc tgg gag cat gcc gcc aag ctc tac gag gac gtc ctc   2464
Gln Asp Phe Ser Trp Glu His Ala Ala Lys Leu Tyr Glu Asp Val Leu
            780                 785                 790

ctc aag gcc aag tac cag tgg tgaacgctag ctgctagccg ctccagcccc      2515
Leu Lys Ala Lys Tyr Gln Trp
        795

gcatgcgtgc atgcatgaga gggtggaact gcgcattgcg cccgcaggaa cgtgccatcc 2575

ttctcgatgg gagcgccggc atccgcgagg tgcagtgaca tgagaggtgt gtgtggttga 2635

gacgctgatt ccgatctcga tctggtccgt agcagagtag agcggacgta gggaagcgct 2695

ccttgttgca ggtatatggg aatgttgtca acttggtatt gtagtttgct atgttgtatg 2755

cgttattaca atgttgttac ttattcttgt taagtcggag gcaaagggcg aaagctagct 2815

cacatgaaaa aaaaaaaaaa aaaaaaa                                     2842
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<210> 4
<211> 799
<212> PRT
<213> Triticum aestivum

<400> 4

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Met Ser Ser Ala Val Ala Ser Ala Ala Ser Phe Leu Ala Leu Ala Ser
  1               5                  10                  15

Ala Ser Pro Gly Arg Ser Arg Arg Arg Ala Arg Val Ser Ala Pro Pro
            20                  25                  30
```

```
Pro His Ala Gly Ala Gly Arg Leu His Trp Pro Pro Trp Pro Pro Gln
          35                  40                  45

Arg Thr Ala Arg Asp Gly Gly Val Ala Ala Arg Ala Ala Gly Lys Lys
      50                  55                  60

Asp Ala Arg Val Asp Asp Asp Ala Ala Ser Ala Arg Gln Pro Arg Ala
 65                  70                  75                  80

Arg Arg Gly Gly Ala Ala Thr Lys Val Ala Glu Arg Arg Asp Pro Val
                 85                  90                  95

Lys Thr Leu Asp Arg Asp Ala Ala Glu Gly Gly Ala Pro Ala Pro Pro
            100                 105                 110

Ala Pro Arg Gln Asp Ala Ala Arg Pro Pro Ser Met Asn Gly Thr Pro
        115                 120                 125

Val Asn Gly Glu Asn Lys Ser Thr Gly Gly Gly Gly Ala Thr Lys Asp
    130                 135                 140

Ser Gly Leu Pro Ala Pro Ala Arg Ala Pro His Pro Ser Thr Gln Asn
145                 150                 155                 160

Arg Val Pro Val Asn Gly Glu Asn Lys Ala Asn Val Ala Ser Pro Pro
                165                 170                 175

Thr Ser Ile Ala Glu Val Val Ala Pro Asp Ser Ala Ala Thr Ile Ser
            180                 185                 190

Ile Ser Asp Lys Ala Pro Glu Ser Val Val Pro Ala Glu Lys Pro Pro
        195                 200                 205

Pro Ser Ser Gly Ser Asn Phe Val Val Ser Ala Ser Ala Pro Arg Leu
    210                 215                 220

Asp Ile Asp Ser Asp Val Glu Pro Glu Leu Lys Lys Gly Ala Val Ile
225                 230                 235                 240

Val Glu Glu Ala Pro Asn Pro Lys Ala Leu Ser Pro Pro Ala Ala Pro
                245                 250                 255

Ala Val Gln Glu Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe Glu
            260                 265                 270

Glu Pro Val Glu Ala Lys Asp Asp Gly Trp Ala Val Ala Asp Asp Ala
        275                 280                 285

Gly Ser Phe Glu His His Gln Asn His Asp Ser Gly Pro Leu Ala Gly
    290                 295                 300

Glu Asn Val Met Asn Val Val Val Val Ala Ala Glu Cys Ser Pro Trp
305                 310                 315                 320

Cys Lys Thr Gly Gly Leu Gly Asp Val Ala Gly Ala Leu Pro Lys Ala
                325                 330                 335

Leu Ala Lys Arg Gly His Arg Val Met Val Val Val Pro Arg Tyr Gly
            340                 345                 350

Asp Tyr Glu Glu Ala Tyr Asp Val Gly Val Arg Lys Tyr Tyr Lys Ala
        355                 360                 365

Ala Gly Gln Asp Met Glu Val Asn Tyr Phe His Ala Tyr Ile Asp Gly
    370                 375                 380
```

```
Val Asp Phe Val Phe Ile Asp Ala Pro Leu Phe Arg His Arg Gln Glu
385                 390                 395                 400

Asp Ile Tyr Gly Gly Ser Arg Gln Glu Ile Met Lys Arg Met Ile Leu
                405                 410                 415

Phe Cys Lys Ala Ala Val Glu Val Pro Trp His Val Pro Cys Gly Gly
            420                 425                 430

Val Pro Tyr Gly Asp Gly Asn Leu Val Phe Ile Ala Asn Asp Trp His
        435                 440                 445

Thr Ala Leu Leu Pro Val Tyr Leu Lys Ala Tyr Tyr Arg Asp His Gly
    450                 455                 460

Leu Met Gln Tyr Thr Arg Ser Ile Met Val Ile His Asn Ile Ala His
465                 470                 475                 480

Gln Gly Arg Gly Pro Val Asp Glu Phe Pro Phe Thr Glu Leu Pro Glu
                485                 490                 495

His Tyr Leu Glu His Phe Arg Leu Tyr Asp Pro Val Gly Gly Glu His
            500                 505                 510

Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met Ala Asp Gln Val Val Val
        515                 520                 525

Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys Thr Val Glu Gly Gly Trp
    530                 535                 540

Gly Leu His Asp Ile Ile Arg Gln Asn Asp Trp Lys Thr Arg Gly Ile
545                 550                 555                 560

Val Asn Gly Ile Asp Asn Met Glu Trp Asn Pro Glu Val Asp Val His
                565                 570                 575

Leu Lys Ser Asp Gly Tyr Thr Asn Phe Ser Leu Gly Thr Leu Asp Ser
            580                 585                 590

Gly Lys Arg Gln Cys Lys Glu Ala Leu Gln Arg Glu Leu Gly Leu Gln
        595                 600                 605

Val Arg Ala Asp Val Pro Leu Leu Gly Phe Ile Gly Arg Leu Asp Gly
    610                 615                 620

Gln Lys Gly Val Glu Ile Ile Ala Asp Ala Met Pro Trp Ile Val Ser
625                 630                 635                 640

Gln Asp Val Gln Leu Val Met Leu Gly Thr Gly Arg His Asp Leu Glu
                645                 650                 655

Ser Met Leu Arg His Phe Glu Arg Glu His His Asp Lys Val Arg Gly
            660                 665                 670

Trp Val Gly Phe Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly Ala
        675                 680                 685

Asp Ala Leu Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln
    690                 695                 700

Leu Tyr Ala Met Ala Tyr Gly Thr Val Pro Val Val His Ala Val Gly
705                 710                 715                 720

Gly Val Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly
```

```
                725                 730                 735

Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala His Lys Leu Ile Glu Ala
            740                 745                 750

Leu Gly His Cys Leu Arg Thr Tyr Arg Asp Tyr Lys Glu Ser Trp Arg
        755                 760                 765

Gly Leu Gln Glu Arg Gly Met Ser Gln Asp Phe Ser Trp Glu His Ala
    770                 775                 780

Ala Lys Leu Tyr Glu Asp Val Leu Leu Lys Ala Lys Tyr Gln Trp
785                 790                 795
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<210> 5
<211> 2107
<212> DNA
<213> Triticum aestivum

<220>
<221> CDS
<222> (1)..(1791)

<400> 5

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Pro Ala Glu Lys Thr Pro Pro Ser Ser Gly Ser Asn Phe Glu Ser Ser
  1               5                  10                  15

gcc tct gct ccc ggg tct gac act gtc agc gac gtg gaa caa gaa ctg   96
Ala Ser Ala Pro Gly Ser Asp Thr Val Ser Asp Val Glu Gln Glu Leu
            20                  25                  30

aag aag ggt gcg gtc gtt gtc gaa gaa gct cca aag cca aag gct ctt   144
Lys Lys Gly Ala Val Val Val Glu Glu Ala Pro Lys Pro Lys Ala Leu
        35                  40                  45

tcg ccg cct gca gcc ccc gct gta caa gaa gac ctt tgg gat ttc aag   192
Ser Pro Pro Ala Ala Pro Ala Val Gln Glu Asp Leu Trp Asp Phe Lys
    50                  55                  60

aaa tac att ggt ttc gag gag ccc gtg gag gcc aag gat gat ggc cgg   240
Lys Tyr Ile Gly Phe Glu Glu Pro Val Glu Ala Lys Asp Asp Gly Arg
65                  70                  75                  80

gct gtc gca gat gat gcg ggc tcc ttt gaa cac cac cag aat cac gac   288
Ala Val Ala Asp Asp Ala Gly Ser Phe Glu His His Gln Asn His Asp
                85                  90                  95

tcc gga cct ttg gca ggg gag aat gtc atg aac gtg gtc gtc gtg gct   336
Ser Gly Pro Leu Ala Gly Glu Asn Val Met Asn Val Val Val Val Ala
            100                 105                 110

gct gag tgt tct ccc tgg tgc aaa aca ggt ggt ctg gga gat gtt gcg   384
Ala Glu Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp Val Ala
        115                 120                 125

ggt gct ctg ccc aag gct ttg gca aag aga gga cat cgt gtt atg gtt   432
Gly Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His Arg Val Met Val
    130                 135                 140

gtg gta cca agg tat ggg gac tat gaa gaa cct acg gat gtc gga gtc   480
Val Val Pro Arg Tyr Gly Asp Tyr Glu Glu Pro Thr Asp Val Gly Val
145                 150                 155                 160
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cga aaa tac tac aag gct gct gga cag gat atg gaa gtg aat tat ttc   528
Arg Lys Tyr Tyr Lys Ala Ala Gly Gln Asp Met Glu Val Asn Tyr Phe
                165                 170                 175

cat gct tat atc gat gga gtt gat ttt gtg ttc att gac gct cct ctc   576
His Ala Tyr Ile Asp Gly Val Asp Phe Val Phe Ile Asp Ala Pro Leu
            180                 185                 190

ttc cga cac cga gag gaa gac att tat ggg ggc agc aga cag gaa att   624
Phe Arg His Arg Glu Glu Asp Ile Tyr Gly Gly Ser Arg Gln Glu Ile
        195                 200                 205

atg aag cgc atg att ttg ttc tgc aag gcc gct gtt gag gtt cca tgg   672
Met Lys Arg Met Ile Leu Phe Cys Lys Ala Ala Val Glu Val Pro Trp
    210                 215                 220

cac gtt cca tgc ggc ggt gtc cct tat ggg gat gga aat ctg gtg ttt   720
His Val Pro Cys Gly Gly Val Pro Tyr Gly Asp Gly Asn Leu Val Phe
225                 230                 235                 240

att gca aat gat tgg cac acg gca ctc ctg cct gtc tat ctg aaa gca   768
Ile Ala Asn Asp Trp His Thr Ala Leu Leu Pro Val Tyr Leu Lys Ala
                245                 250                 255

tat tac agg gac cat ggt ttg atg cag tac act cgg tcc att atg gtg   816
Tyr Tyr Arg Asp His Gly Leu Met Gln Tyr Thr Arg Ser Ile Met Val
            260                 265                 270

ata cat aac atc gct cac cag ggc cgt ggc cct gta gat gaa ttc ccg   864
Ile His Asn Ile Ala His Gln Gly Arg Gly Pro Val Asp Glu Phe Pro
        275                 280                 285

ttc acc gag ttg cct gag cac tac ctg gaa cac ttc aga ctg tac gac   912
Phe Thr Glu Leu Pro Glu His Tyr Leu Glu His Phe Arg Leu Tyr Asp
    290                 295                 300

ccc gtg ggt ggt gaa cac gcc aac tac ttc gcc gcc ggc ctg aag atg   960
Pro Val Gly Gly Glu His Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met
305                 310                 315                 320

gcg gac cag gtt gtc gtg gtg agc ccc ggg tac ctg tgg gag ctg aag  1008
Ala Asp Gln Val Val Val Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys
                325                 330                 335

acg gtg gag ggc ggc tgg ggg ctt cac gac atc ata cgg cag aac gac  1056
Thr Val Glu Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln Asn Asp
            340                 345                 350

tgg aag acc cgc ggc atc gtc aac ggc atc gac aac atg gag tgg aac  1104
Trp Lys Thr Arg Gly Ile Val Asn Gly Ile Asp Asn Met Glu Trp Asn
        355                 360                 365

ccc gag gtg gac gcc cac ctc aag tcg gac ggc tac acc aac ttc tcc  1152
Pro Glu Val Asp Ala His Leu Lys Ser Asp Gly Tyr Thr Asn Phe Ser
    370                 375                 380

ctg agg acg ctg gac tcc ggc aag cgg cag tgc aag gag gcc ctg cag  1200
Leu Arg Thr Leu Asp Ser Gly Lys Arg Gln Cys Lys Glu Ala Leu Gln
385                 390                 395                 400

cgc gag ctg ggc ctg cag gtc cgc gcc gac gtg ccg ctg ctc ggc ttc  1248
Arg Glu Leu Gly Leu Gln Val Arg Ala Asp Val Pro Leu Leu Gly Phe
                405                 410                 415

atc ggc cgc ctg gac ggg cag aag ggc gtg gag atc atc gcg gac gcc  1296
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Ile Gly Arg Leu Asp Gly Gln Lys Gly Val Glu Ile Ile Ala Asp Ala
            420                 425                 430

atg ccc tgg atc gtg agc cag gac gtg cag ctg gtg atg ctg ggc acc   1344
Met Pro Trp Ile Val Ser Gln Asp Val Gln Leu Val Met Leu Gly Thr
        435                 440                 445

ggg cgc cac gac ctg gag agc atg ctg cag cac ttc gag cgg gag cac   1392
Gly Arg His Asp Leu Glu Ser Met Leu Gln His Phe Glu Arg Glu His
    450                 455                 460

cac gac aag gtg cgc ggg tgg gtg ggg ttc tcc gtg cgc ctg gcg cac   1440
His Asp Lys Val Arg Gly Trp Val Gly Phe Ser Val Arg Leu Ala His
465                 470                 475                 480

cgg atc acg gcg ggg gcg gac gcg ctc ctc atg ccc tcc cgg ttc gtg   1488
Arg Ile Thr Ala Gly Ala Asp Ala Leu Leu Met Pro Ser Arg Phe Val
                485                 490                 495

ccg tgc ggg ctg aac cag ctc tac gcc atg gcc tac ggc acc gtc ccc   1536
Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr Val Pro
            500                 505                 510

gtc gtg cac gcc gtc ggc ggc ctc agg gac acc gtg ccg ccg ttc gac   1584
Val Val His Ala Val Gly Gly Leu Arg Asp Thr Val Pro Pro Phe Asp
        515                 520                 525

ccc ttc aac cac tcc ggg ctc ggg tgg acg ttc gac cgc gcc gag gcg   1632
Pro Phe Asn His Ser Gly Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala
    530                 535                 540

cac aag ctg atc gag gcg ctc ggg cac tgc ctc cgc acc tac cga gac   1680
His Lys Leu Ile Glu Ala Leu Gly His Cys Leu Arg Thr Tyr Arg Asp
545                 550                 555                 560

ttc aag gag agc tgg agg gcc ctc cag gag cgc ggc atg tcg cag gac   1728
Phe Lys Glu Ser Trp Arg Ala Leu Gln Glu Arg Gly Met Ser Gln Asp
                565                 570                 575

ttc agc tgg gag cac gcc gcc aag ctc tac gag gac gtc ctc gtc aag   1776
Phe Ser Trp Glu His Ala Ala Lys Leu Tyr Glu Asp Val Leu Val Lys
            580                 585                 590

gcc aag tac cag tgg tgaacgctag ctgctagccg ctccagcccc gcatgcgtgc   1831
Ala Lys Tyr Gln Trp
        595

atgacaggat ggaactgcat tgcgcacgca ggaaagtgcc atggagcgcc ggcatccgcg 1891

aagtacagtg acatgaggtg tgtgtggttg agacgctgat tccaatccgg cccgtagcag 1951

agtagagcgg aggtatatgg gaatcttaac ttggtattgt aatttgttat gttgtgtgca 2011

ttattacaat gttgttactt attcttgtta agtcggaggc caagggcgaa agctagctca 2071

catgtctgat ggatgcaaaa aaaaaaaaaa aaaaaa                           2107
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<210> 6
<211> 597
<212> PRT
<213> Triticum aestivum

<400> 6
Pro Ala Glu Lys Thr Pro Pro Ser Ser Gly Ser Asn Phe Glu Ser Ser

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1               5                  10                  15

Ala Ser Ala Pro Gly Ser Asp Thr Val Ser Asp Val Glu Gln Glu Leu
            20                  25                  30

Lys Lys Gly Ala Val Val Val Glu Glu Ala Pro Lys Pro Lys Ala Leu
        35                  40                  45

Ser Pro Pro Ala Ala Pro Ala Val Gln Glu Asp Leu Trp Asp Phe Lys
    50                  55                  60

Lys Tyr Ile Gly Phe Glu Glu Pro Val Glu Ala Lys Asp Asp Gly Arg
65                  70                  75                  80

Ala Val Ala Asp Asp Ala Gly Ser Phe Glu His His Gln Asn His Asp
                85                  90                  95

Ser Gly Pro Leu Ala Gly Glu Asn Val Met Asn Val Val Val Val Ala
            100                 105                 110

Ala Glu Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp Val Ala
        115                 120                 125

Gly Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His Arg Val Met Val
    130                 135                 140

Val Val Pro Arg Tyr Gly Asp Tyr Glu Glu Pro Thr Asp Val Gly Val
145                 150                 155                 160

Arg Lys Tyr Tyr Lys Ala Ala Gly Gln Asp Met Glu Val Asn Tyr Phe
                165                 170                 175

His Ala Tyr Ile Asp Gly Val Asp Phe Val Phe Ile Asp Ala Pro Leu
            180                 185                 190

Phe Arg His Arg Glu Glu Asp Ile Tyr Gly Gly Ser Arg Gln Glu Ile
        195                 200                 205

Met Lys Arg Met Ile Leu Phe Cys Lys Ala Ala Val Glu Val Pro Trp
    210                 215                 220

His Val Pro Cys Gly Gly Val Pro Tyr Gly Asp Gly Asn Leu Val Phe
225                 230                 235                 240

Ile Ala Asn Asp Trp His Thr Ala Leu Leu Pro Val Tyr Leu Lys Ala
                245                 250                 255

Tyr Tyr Arg Asp His Gly Leu Met Gln Tyr Thr Arg Ser Ile Met Val
            260                 265                 270

Ile His Asn Ile Ala His Gln Gly Arg Gly Pro Val Asp Glu Phe Pro
        275                 280                 285

Phe Thr Glu Leu Pro Glu His Tyr Leu Glu His Phe Arg Leu Tyr Asp
    290                 295                 300

Pro Val Gly Gly Glu His Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met
305                 310                 315                 320

Ala Asp Gln Val Val Val Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys
                325                 330                 335

Thr Val Glu Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln Asn Asp
            340                 345                 350
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Trp Lys Thr Arg Gly Ile Val Asn Gly Ile Asp Asn Met Glu Trp Asn
        355                 360                 365

Pro Glu Val Asp Ala His Leu Lys Ser Asp Gly Tyr Thr Asn Phe Ser
    370                 375                 380

Leu Arg Thr Leu Asp Ser Gly Lys Arg Gln Cys Lys Glu Ala Leu Gln
385                 390                 395                 400

Arg Glu Leu Gly Leu Gln Val Arg Ala Asp Val Pro Leu Leu Gly Phe
                405                 410                 415

Ile Gly Arg Leu Asp Gly Gln Lys Gly Val Glu Ile Ile Ala Asp Ala
            420                 425                 430

Met Pro Trp Ile Val Ser Gln Asp Val Gln Leu Val Met Leu Gly Thr
        435                 440                 445

Gly Arg His Asp Leu Glu Ser Met Leu Gln His Phe Glu Arg Glu His
    450                 455                 460

His Asp Lys Val Arg Gly Trp Val Gly Phe Ser Val Arg Leu Ala His
465                 470                 475                 480

Arg Ile Thr Ala Gly Ala Asp Ala Leu Leu Met Pro Ser Arg Phe Val
                485                 490                 495

Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr Val Pro
            500                 505                 510

Val Val His Ala Val Gly Gly Leu Arg Asp Thr Val Pro Pro Phe Asp
        515                 520                 525

Pro Phe Asn His Ser Gly Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala
    530                 535                 540

His Lys Leu Ile Glu Ala Leu Gly His Cys Leu Arg Thr Tyr Arg Asp
545                 550                 555                 560

Phe Lys Glu Ser Trp Arg Ala Leu Gln Glu Arg Gly Met Ser Gln Asp
                565                 570                 575

Phe Ser Trp Glu His Ala Ala Lys Leu Tyr Glu Asp Val Leu Val Lys
            580                 585                 590

Ala Lys Tyr Gln Trp
        595
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<210> 7
<211> 5346
<212> DNA
<213> Triticum aestivum

<220>
<221> CDS
<222> (29)..(4912)

<400> 7

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cggcacgagg tttagtaggt tccgggaa atg gag atg tct ctc tgg cca cgg    52
                               Met Glu Met Ser Leu Trp Pro Arg
                                 1               5

agc ccc ctg tgc cct cgg agc agg cag ccg ctc gtc gtc gtc cgg ccg   100
Ser Pro Leu Cys Pro Arg Ser Arg Gln Pro Leu Val Val Val Arg Pro
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     10                  15                  20

gcc ggc cgc ggc ggc ctc acg cag cct ttt ttg atg aat ggc aga ttt   148
Ala Gly Arg Gly Gly Leu Thr Gln Pro Phe Leu Met Asn Gly Arg Phe
 25                  30                  35                  40

act cga agc agg acc ctt cga tgc atg gta gca agt tca gat cct cct   196
Thr Arg Ser Arg Thr Leu Arg Cys Met Val Ala Ser Ser Asp Pro Pro
                 45                  50                  55

aat agg aaa tca aga agg atg gta cca cct cag gtt aaa gtc att tct   244
Asn Arg Lys Ser Arg Arg Met Val Pro Pro Gln Val Lys Val Ile Ser
             60                  65                  70

tct aga gga tat acg aca aga ctc att gtt gaa cca agc aac gag aat   292
Ser Arg Gly Tyr Thr Thr Arg Leu Ile Val Glu Pro Ser Asn Glu Asn
         75                  80                  85

aca gaa cac aat aat cgg gat gaa gaa act ctt gat aca tac aat gcg   340
Thr Glu His Asn Asn Arg Asp Glu Glu Thr Leu Asp Thr Tyr Asn Ala
     90                  95                 100

cta tta agt acc gag aca gca gaa tgg aca gat aat aga gaa gcc gag   388
Leu Leu Ser Thr Glu Thr Ala Glu Trp Thr Asp Asn Arg Glu Ala Glu
105                 110                 115                 120

act gct aaa gcg gac tcg tcg caa aat gct tta agc agt tct ata att   436
Thr Ala Lys Ala Asp Ser Ser Gln Asn Ala Leu Ser Ser Ser Ile Ile
                125                 130                 135

ggg gaa gtg gat gtg gcg gat gaa gat ata ctt gcg gct gat ctg aca   484
Gly Glu Val Asp Val Ala Asp Glu Asp Ile Leu Ala Ala Asp Leu Thr
            140                 145                 150

gtg tat tca ttg agc agt gta atg aag aag gaa gtg gat gca gcg gac   532
Val Tyr Ser Leu Ser Ser Val Met Lys Lys Glu Val Asp Ala Ala Asp
        155                 160                 165

aaa gct aga gtt aaa gaa gac gca ttt gag ctg gat ttg cca gca act   580
Lys Ala Arg Val Lys Glu Asp Ala Phe Glu Leu Asp Leu Pro Ala Thr
    170                 175                 180

aca ttg aga agt gtg ata gta gat gtg atg gat cat aat ggg act gta   628
Thr Leu Arg Ser Val Ile Val Asp Val Met Asp His Asn Gly Thr Val
185                 190                 195                 200

caa gag aca ttg aga agt gtg ata gta gat gtg atg gat cat aat ggg   676
Gln Glu Thr Leu Arg Ser Val Ile Val Asp Val Met Asp His Asn Gly
                205                 210                 215

act gta caa gag aca ttg aga agt gtg ata gta gat gtg atg gat gat   724
Thr Val Gln Glu Thr Leu Arg Ser Val Ile Val Asp Val Met Asp Asp
            220                 225                 230

gcg gcg gac aaa gct aga gtt gaa gaa gac gta ttt gag ctg gat ttg   772
Ala Ala Asp Lys Ala Arg Val Glu Glu Asp Val Phe Glu Leu Asp Leu
        235                 240                 245

tca gga aat att tca agc agt gcg acg acc gtg gaa cta gat gcg gtt   820
Ser Gly Asn Ile Ser Ser Ser Ala Thr Thr Val Glu Leu Asp Ala Val
    250                 255                 260

gac gaa gtc ggg cct gtt caa gac aaa ttt gag gcg acc tca tca gga   868
Asp Glu Val Gly Pro Val Gln Asp Lys Phe Glu Ala Thr Ser Ser Gly
265                 270                 275                 280
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agt caa gat ctt tcg gct gtt agt ctc cct aaa caa aac gta cca att   1684
Ser Gln Asp Leu Ser Ala Val Ser Leu Pro Lys Gln Asn Val Pro Ile
            540                 545                 550

gtt ggt acg tcg aga gag ggt caa aca aag caa gtt cct gtt gtt gat   1732
Val Gly Thr Ser Arg Glu Gly Gln Thr Lys Gln Val Pro Val Val Asp
        555                 560                 565

aga cag gat gca ttg tat gtg aat gga ctg gaa gct aag gag gga gat   1780
Arg Gln Asp Ala Leu Tyr Val Asn Gly Leu Glu Ala Lys Glu Gly Asp
    570                 575                 580

cac aca tcc gag aaa act gat gag gat gcg ctt cat gta aag ttt aat   1828
His Thr Ser Glu Lys Thr Asp Glu Asp Ala Leu His Val Lys Phe Asn
585                 590                 595                 600

gtt gac aat gtg ttg cgg aag cat cag gca gat aga acc caa gca gtg   1876
Val Asp Asn Val Leu Arg Lys His Gln Ala Asp Arg Thr Gln Ala Val
                605                 610                 615

gaa aag aaa act tgg aag aaa gtt gat gag gaa cat ctt tac atg act   1924
Glu Lys Lys Thr Trp Lys Lys Val Asp Glu Glu His Leu Tyr Met Thr
            620                 625                 630

gaa cat cag aaa cgt gct gcc gaa gga cag atg gta gtt aac gag gat   1972
Glu His Gln Lys Arg Ala Ala Glu Gly Gln Met Val Val Asn Glu Asp
        635                 640                 645

gag ctt tct ata act gaa att gga atg ggg aga ggt gat aaa att cag   2020
Glu Leu Ser Ile Thr Glu Ile Gly Met Gly Arg Gly Asp Lys Ile Gln
    650                 655                 660

cat gtg ctt tct gag gaa gag ctt tca tgg tct gaa gat gaa gtg cag   2068
His Val Leu Ser Glu Glu Glu Leu Ser Trp Ser Glu Asp Glu Val Gln
665                 670                 675                 680

tta att gag gat gat gga caa tat gaa gtt gac gag acc tct gtg tcc   2116
Leu Ile Glu Asp Asp Gly Gln Tyr Glu Val Asp Glu Thr Ser Val Ser
                685                 690                 695

gtt aac gtt gaa caa gat atc cag ggg tca cca cag gat gtt gtg gat   2164
Val Asn Val Glu Gln Asp Ile Gln Gly Ser Pro Gln Asp Val Val Asp
            700                 705                 710

ccg caa gca cta aag gtg atg ctg caa gaa ctc gct gag aaa aat tat   2212
Pro Gln Ala Leu Lys Val Met Leu Gln Glu Leu Ala Glu Lys Asn Tyr
        715                 720                 725

tcg atg agg aac aag ctg ttt gtt ttt cca gag gta gtg aaa gct gat   2260
Ser Met Arg Asn Lys Leu Phe Val Phe Pro Glu Val Val Lys Ala Asp
    730                 735                 740

tca gtt att gat ctt tat tta aat cgt gac cta aca gct ttg gcg aat   2308
Ser Val Ile Asp Leu Tyr Leu Asn Arg Asp Leu Thr Ala Leu Ala Asn
745                 750                 755                 760

gaa ccc gat gtc gtc atc aaa gga gca ttc aat ggt tgg aaa tgg agg   2356
Glu Pro Asp Val Val Ile Lys Gly Ala Phe Asn Gly Trp Lys Trp Arg
                765                 770                 775

ctt ttc act gaa aga ttg cac aag agt gac ctt gga ggg gtt tgg tgg   2404
Leu Phe Thr Glu Arg Leu His Lys Ser Asp Leu Gly Gly Val Trp Trp
            780                 785                 790

tct tgc aaa ctg tac ata ccc aag gag gcc tac aga tta gac ttt gtg   2452
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aat gtt tca aac agt gca acg gta cgg gaa gtg gat gca agt gat gaa   916
Asn Val Ser Asn Ser Ala Thr Val Arg Glu Val Asp Ala Ser Asp Glu
                285                 290                 295

gct ggg aat gat caa ggc ata ttt aga gca gat ttg tca gga aat gtt   964
Ala Gly Asn Asp Gln Gly Ile Phe Arg Ala Asp Leu Ser Gly Asn Val
            300                 305                 310

ttt tca agc agt aca aca gtg gaa gtg ggt gca gtg gat gaa gct ggg   1012
Phe Ser Ser Ser Thr Thr Val Glu Val Gly Ala Val Asp Glu Ala Gly
        315                 320                 325

tct ata aag gac agg ttt gag acg gat tcg tca gga aat gtt tca aca   1060
Ser Ile Lys Asp Arg Phe Glu Thr Asp Ser Ser Gly Asn Val Ser Thr
    330                 335                 340

agt gcg ccg atg tgg gat gca att gat gaa acc gtg gct gat caa gac   1108
Ser Ala Pro Met Trp Asp Ala Ile Asp Glu Thr Val Ala Asp Gln Asp
345                 350                 355                 360

aca ttt gag gcg gat ttg tcg gga aat gct tca agc tgc gca aca tac   1156
Thr Phe Glu Ala Asp Leu Ser Gly Asn Ala Ser Ser Cys Ala Thr Tyr
                365                 370                 375

aga gaa gtg gat gat gtg gtg gat gaa act aga tca gaa gag gaa aca   1204
Arg Glu Val Asp Asp Val Val Asp Glu Thr Arg Ser Glu Glu Glu Thr
            380                 385                 390

ttt gca atg gat ttg ttt gca agt gaa tca ggc cat gag aaa cat atg   1252
Phe Ala Met Asp Leu Phe Ala Ser Glu Ser Gly His Glu Lys His Met
        395                 400                 405

gca gtg gat tat gtg ggt gaa gct acc gat gaa gaa gag act tac caa   1300
Ala Val Asp Tyr Val Gly Glu Ala Thr Asp Glu Glu Glu Thr Tyr Gln
    410                 415                 420

cag caa tat cca gta ccg tct tca ttc tct atg tgg gac aag gct att   1348
Gln Gln Tyr Pro Val Pro Ser Ser Phe Ser Met Trp Asp Lys Ala Ile
425                 430                 435                 440

gct aaa aca ggt gta agt ttg aat cct gag ctg cga ctt gtc agg gtt   1396
Ala Lys Thr Gly Val Ser Leu Asn Pro Glu Leu Arg Leu Val Arg Val
                445                 450                 455

gaa gaa caa ggc aaa gta aat ttt agt gat aaa aaa gac ctg tca att   1444
Glu Glu Gln Gly Lys Val Asn Phe Ser Asp Lys Lys Asp Leu Ser Ile
            460                 465                 470

gat gat tta cca gga caa aac caa tcg atc att ggt tcc tat aaa caa   1492
Asp Asp Leu Pro Gly Gln Asn Gln Ser Ile Ile Gly Ser Tyr Lys Gln
        475                 480                 485

gat aaa tca att gct gat gtt gcg gga ccg acc caa tca att ttt ggt   1540
Asp Lys Ser Ile Ala Asp Val Ala Gly Pro Thr Gln Ser Ile Phe Gly
    490                 495                 500

tct agt aaa caa cac cgg tca att gtt gct ttc ccc aaa caa aac cag   1588
Ser Ser Lys Gln His Arg Ser Ile Val Ala Phe Pro Lys Gln Asn Gln
505                 510                 515                 520

tca att gtt agt gtc act gag caa aag cag tcc ata gtt gga ttc cgt   1636
Ser Ile Val Ser Val Thr Glu Gln Lys Gln Ser Ile Val Gly Phe Arg
                525                 530                 535
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    1050                1055                1060

ctg ctt tcc cag aaa cac att gtt tat acc gaa ccg ctt gaa ata cgt   3268
Leu Leu Ser Gln Lys His Ile Val Tyr Thr Glu Pro Leu Glu Ile Arg
1065              1070                1075                1080

gcc gga acc aca gtg gat gtg cta tac aat ccc tct aac aca gtg cta   3316
Ala Gly Thr Thr Val Asp Val Leu Tyr Asn Pro Ser Asn Thr Val Leu
                1085                1090                1095

aat gga aag tcg gag ggt tgg ttt aga tgc tcc ttt aac ctt tgg atg   3364
Asn Gly Lys Ser Glu Gly Trp Phe Arg Cys Ser Phe Asn Leu Trp Met
           1100                1105                1110

cat tca agt ggg gca ttg cca ccc cag aag atg gtg aaa tca ggg gat   3412
His Ser Ser Gly Ala Leu Pro Pro Gln Lys Met Val Lys Ser Gly Asp
       1115                1120                1125

ggg ccg ctc tta aaa gca aca gtt gat gtt cca ccg gat gcc tat atg   3460
Gly Pro Leu Leu Lys Ala Thr Val Asp Val Pro Pro Asp Ala Tyr Met
   1130                1135                1140

atg gac ttt gtt ttc tcc gag tgg gaa gaa gat ggg atc tat gac aac   3508
Met Asp Phe Val Phe Ser Glu Trp Glu Glu Asp Gly Ile Tyr Asp Asn
1145                1150                1155                1160

agg aat ggg atg gac tat cat att cct gtt tct gat tca att gaa aca   3556
Arg Asn Gly Met Asp Tyr His Ile Pro Val Ser Asp Ser Ile Glu Thr
                1165                1170                1175

gag aat tac atg cgt att atc cac att gcc gtt gag atg gcc ccc gtt   3604
Glu Asn Tyr Met Arg Ile Ile His Ile Ala Val Glu Met Ala Pro Val
           1180                1185                1190

gca aag gtt gga ggt ctt ggg gat gtt gtt aca agt ctt tca cgt gcc   3652
Ala Lys Val Gly Gly Leu Gly Asp Val Val Thr Ser Leu Ser Arg Ala
       1195                1200                1205

att caa gat cta gga cat act gtc gag gtt att ctc ccg aag tac gac   3700
Ile Gln Asp Leu Gly His Thr Val Glu Val Ile Leu Pro Lys Tyr Asp
   1210                1215                1220

tgt ttg aac caa agc agt gtc aag gat tta cat tta tat caa agt ttt   3748
Cys Leu Asn Gln Ser Ser Val Lys Asp Leu His Leu Tyr Gln Ser Phe
1225                1230                1235                1240

tct tgg ggt ggt aca gaa ata aaa gta tgg gtt gga cga gtc gaa gac   3796
Ser Trp Gly Gly Thr Glu Ile Lys Val Trp Val Gly Arg Val Glu Asp
                1245                1250                1255

ctg acc gtt tac ttc ctg gaa cct caa aat ggg atg ttt ggc gtt gga   3844
Leu Thr Val Tyr Phe Leu Glu Pro Gln Asn Gly Met Phe Gly Val Gly
           1260                1265                1270

tgt gta tat gga agg aat gat gac cgc aga ttt ggg ttc ttc tgt cat   3892
Cys Val Tyr Gly Arg Asn Asp Asp Arg Arg Phe Gly Phe Phe Cys His
       1275                1280                1285

tct gct cta gag ttt atc ctc cag aat gaa ttt tct cca cat ata ata   3940
Ser Ala Leu Glu Phe Ile Leu Gln Asn Glu Phe Ser Pro His Ile Ile
   1290                1295                1300

cat tgc cat gat tgg tca agt gct ccg gtc gcc tgg cta tat aag gaa   3988
His Cys His Asp Trp Ser Ser Ala Pro Val Ala Trp Leu Tyr Lys Glu
1305                1310                1315                1320
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Ser Cys Lys Leu Tyr Ile Pro Lys Glu Ala Tyr Arg Leu Asp Phe Val
        795                 800                 805

ttc ttc aac ggt cgc acg gtc tat gag aac aat ggc aac aat gat ttc   2500
Phe Phe Asn Gly Arg Thr Val Tyr Glu Asn Asn Gly Asn Asn Asp Phe
    810                 815                 820

tgt ata gga ata gaa ggc act atg aat gaa gat ctg ttt gag gat ttc   2548
Cys Ile Gly Ile Glu Gly Thr Met Asn Glu Asp Leu Phe Glu Asp Phe
825                 830                 835                 840

ttg gtt aaa gaa aag caa agg gag ctt gag aaa ctt gcc atg gaa gaa   2596
Leu Val Lys Glu Lys Gln Arg Glu Leu Glu Lys Leu Ala Met Glu Glu
                845                 850                 855

gct gaa agg agg aca cag act gaa gaa cag cgg cga aga aag gaa gca   2644
Ala Glu Arg Arg Thr Gln Thr Glu Glu Gln Arg Arg Arg Lys Glu Ala
            860                 865                 870

agg gct gca gat gaa gct gtc agg gca caa gcg aag gcc gag ata gag   2692
Arg Ala Ala Asp Glu Ala Val Arg Ala Gln Ala Lys Ala Glu Ile Glu
        875                 880                 885

atc aag aag aaa aaa ttg caa agt atg ttg agt ttg gcc aga aca tgt   2740
Ile Lys Lys Lys Lys Leu Gln Ser Met Leu Ser Leu Ala Arg Thr Cys
    890                 895                 900

gtt gat aat ttg tgg tac ata gag gct agc aca gat aca aga gga gat   2788
Val Asp Asn Leu Trp Tyr Ile Glu Ala Ser Thr Asp Thr Arg Gly Asp
905                 910                 915                 920

act atc agg tta tat tat aac aga aac tcg agg cca ctt gcg cat agt   2836
Thr Ile Arg Leu Tyr Tyr Asn Arg Asn Ser Arg Pro Leu Ala His Ser
                925                 930                 935

act gag att tgg atg cat ggt ggt tac aac aat tgg aca gat gga ctc   2884
Thr Glu Ile Trp Met His Gly Gly Tyr Asn Asn Trp Thr Asp Gly Leu
            940                 945                 950

tct att gtt gaa agc ttt gtc aag tgc aat gac aaa gac ggc gat tgg   2932
Ser Ile Val Glu Ser Phe Val Lys Cys Asn Asp Lys Asp Gly Asp Trp
        955                 960                 965

tgg tat gca gat gtt att cca cct gaa aag gca ctt gtg ttg gac tgg   2980
Trp Tyr Ala Asp Val Ile Pro Pro Glu Lys Ala Leu Val Leu Asp Trp
    970                 975                 980

gtt ttt gct gat ggg cca gct ggg aat gca agg aac tat gac aac aat   3028
Val Phe Ala Asp Gly Pro Ala Gly Asn Ala Arg Asn Tyr Asp Asn Asn
985                 990                 995                1000

gct cga caa gat ttc cat gct att ctt ccg aac aac aat gta acc gag   3076
Ala Arg Gln Asp Phe His Ala Ile Leu Pro Asn Asn Asn Val Thr Glu
                1005                1010                1015

gaa ggc ttc tgg gcg caa gag gag caa aac atc tat aca agg ctt ctg   3124
Glu Gly Phe Trp Ala Gln Glu Glu Gln Asn Ile Tyr Thr Arg Leu Leu
            1020                1025                1030

caa gaa agg aga gaa aag gaa gaa acc atg aaa aga aag gct gag aga   3172
Gln Glu Arg Arg Glu Lys Glu Glu Thr Met Lys Arg Lys Ala Glu Arg
        1035                1040                1045

agt gca aat atc aaa gct gag atg aag gca aaa act atg cga agg ttt   3220
Ser Ala Asn Ile Lys Ala Glu Met Lys Ala Lys Thr Met Arg Arg Phe
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aat ggc gtg gat tat gcc ctc aac aga gca atc ggc gct tgg ttc gat   4804
Asn Gly Val Asp Tyr Ala Leu Asn Arg Ala Ile Gly Ala Trp Phe Asp
            1580                1585                1590

gcc cgt gat tgg ttc cac tcc ctg tgt aag agg gtc atg gag caa gac   4852
Ala Arg Asp Trp Phe His Ser Leu Cys Lys Arg Val Met Glu Gln Asp
        1595                1600                1605

tgg tcg tgg aac cgg ccc gca ctg gac tac att gaa ttg tac cat gcc   4900
Trp Ser Trp Asn Arg Pro Ala Leu Asp Tyr Ile Glu Leu Tyr His Ala
    1610                1615                1620

gct cga aaa ttc tgacacccaa ctgaaccaat gacaagaaca agcgcattgt       4952
Ala Arg Lys Phe
1625

gggatcgact agtcatacag ggctgtgcag atcgtcttgc ttcagttagt gccctcttca 5012

gttagttcca agcgcactac agtcgtacat agctgaggat cctcttgcct cctaccaggg 5072

ggaacaaagc agaaatgcat gagtgcattg ggaagacttt tatgtatatt gttaaaaaaa 5132

tttccttttc ttttccttcc ctgcacctgg aaatggttaa gcgcatcgcc gagataagaa 5192

ccgcagtgac attctgtgag tagctttgta tattctctca tcttgtgaaa actaatgttc 5252

atgttaggct gtctgatcat gtggaagctt tgttatatgt tacttatggt atacatcaat 5312

gatatttaca tttgtggaaa aaaaaaaaaa aaaa                             5346
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<212> PRT
<213> Triticum aestivum

<400> 8

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Gln Pro Leu Val Val Val Arg Pro Ala Gly Arg Gly Gly Leu Thr Gln
             20                  25                  30

Pro Phe Leu Met Asn Gly Arg Phe Thr Arg Ser Arg Thr Leu Arg Cys
         35                  40                  45

Met Val Ala Ser Ser Asp Pro Pro Asn Arg Lys Ser Arg Arg Met Val
     50                  55                  60

Pro Pro Gln Val Lys Val Ile Ser Ser Arg Gly Tyr Thr Thr Arg Leu
 65                  70                  75                  80

Ile Val Glu Pro Ser Asn Glu Asn Thr Glu His Asn Asn Arg Asp Glu
                 85                  90                  95

Glu Thr Leu Asp Thr Tyr Asn Ala Leu Leu Ser Thr Glu Thr Ala Glu
            100                 105                 110

Trp Thr Asp Asn Arg Glu Ala Glu Thr Ala Lys Ala Asp Ser Ser Gln
        115                 120                 125

Asn Ala Leu Ser Ser Ser Ile Ile Gly Glu Val Asp Val Ala Asp Glu
    130                 135                 140

Asp Ile Leu Ala Ala Asp Leu Thr Val Tyr Ser Leu Ser Ser Val Met
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cac tat tcc caa tcc aga atg gca agc act cgg gtt gta ttt acc atc   4036
His Tyr Ser Gln Ser Arg Met Ala Ser Thr Arg Val Val Phe Thr Ile
                1325                1330                1335

cac aat ctt gaa ttt gga gca cat tat att ggt aaa gca atg aca tac   4084
His Asn Leu Glu Phe Gly Ala His Tyr Ile Gly Lys Ala Met Thr Tyr
            1340                1345                1350

tgt gat aaa gcc aca act gtt tct cct aca tat tca agg gac gtg gca   4132
Cys Asp Lys Ala Thr Thr Val Ser Pro Thr Tyr Ser Arg Asp Val Ala
        1355                1360                1365

ggc cat ggc gcc att gct cct cat cgt gag aaa ttc tac ggc att ctc   4180
Gly His Gly Ala Ile Ala Pro His Arg Glu Lys Phe Tyr Gly Ile Leu
    1370                1375                1380

aat gga att gat cca gat atc tgg gat ccg tac act gac aat ttt atc   4228
Asn Gly Ile Asp Pro Asp Ile Trp Asp Pro Tyr Thr Asp Asn Phe Ile
1385                1390                1395                1400

ccg gtc cct tat act tgt gag aat gtt gtc gaa ggc aag aga gct gca   4276
Pro Val Pro Tyr Thr Cys Glu Asn Val Val Glu Gly Lys Arg Ala Ala
                1405                1410                1415

aaa agg gcc ttg cag cag aag ttt gga tta cag caa act gat gtc cct   4324
Lys Arg Ala Leu Gln Gln Lys Phe Gly Leu Gln Gln Thr Asp Val Pro
            1420                1425                1430

att gtc gga atc atc acc cgt ctg aca gcc cag aag gga atc cac ctc   4372
Ile Val Gly Ile Ile Thr Arg Leu Thr Ala Gln Lys Gly Ile His Leu
        1435                1440                1445

atc aag cac gca att cac cga act ctc gaa agc aac gga cat gtg gtt   4420
Ile Lys His Ala Ile His Arg Thr Leu Glu Ser Asn Gly His Val Val
    1450                1455                1460

ttg ctt ggt tca gct cca gat cat cga ata caa ggc gat ttt tgc aga   4468
Leu Leu Gly Ser Ala Pro Asp His Arg Ile Gln Gly Asp Phe Cys Arg
1465                1470                1475                1480

ttg gcc gat gct ctt cat ggt gtt tac cat ggt agg gtg aag ctt gtt   4516
Leu Ala Asp Ala Leu His Gly Val Tyr His Gly Arg Val Lys Leu Val
                1485                1490                1495

cta acc tat gat gag cct ctt tct cac ctg ata tac gct ggc tcg gac   4564
Leu Thr Tyr Asp Glu Pro Leu Ser His Leu Ile Tyr Ala Gly Ser Asp
            1500                1505                1510

ttc ata att gtt cct tca atc ttc gaa ccc tgt ggc tta aca caa ctt   4612
Phe Ile Ile Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln Leu
        1515                1520                1525

gtt gcc atg cgt tat gga tcg atc cct ata gtt cgg aaa act gga gga   4660
Val Ala Met Arg Tyr Gly Ser Ile Pro Ile Val Arg Lys Thr Gly Gly
    1530                1535                1540

ctt cac gac aca gtc ttc gac gta gac aat gat aag gac cgg gct cgg   4708
Leu His Asp Thr Val Phe Asp Val Asp Asn Asp Lys Asp Arg Ala Arg
1545                1550                1555                1560

tct ctt ggt ctt gaa cca aat ggg ttc agt ttc gac gga gcc gac agc   4756
Ser Leu Gly Leu Glu Pro Asn Gly Phe Ser Phe Asp Gly Ala Asp Ser
                1565                1570                1575
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Gly Pro Thr Gln Ser Ile Phe Gly Ser Ser Lys Gln His Arg Ser Ile
            500                 505                 510

Val Ala Phe Pro Lys Gln Asn Gln Ser Ile Val Ser Val Thr Glu Gln
        515                 520                 525

Lys Gln Ser Ile Val Gly Phe Arg Ser Gln Asp Leu Ser Ala Val Ser
    530                 535                 540

Leu Pro Lys Gln Asn Val Pro Ile Val Gly Thr Ser Arg Glu Gly Gln
545                 550                 555                 560

Thr Lys Gln Val Pro Val Val Asp Arg Gln Asp Ala Leu Tyr Val Asn
                565                 570                 575

Gly Leu Glu Ala Lys Glu Gly Asp His Thr Ser Glu Lys Thr Asp Glu
            580                 585                 590

Asp Ala Leu His Val Lys Phe Asn Val Asp Asn Val Leu Arg Lys His
        595                 600                 605

Gln Ala Asp Arg Thr Gln Ala Val Glu Lys Lys Thr Trp Lys Lys Val
    610                 615                 620

Asp Glu Glu His Leu Tyr Met Thr Glu His Gln Lys Arg Ala Ala Glu
625                 630                 635                 640

Gly Gln Met Val Val Asn Glu Asp Glu Leu Ser Ile Thr Glu Ile Gly
                645                 650                 655

Met Gly Arg Gly Asp Lys Ile Gln His Val Leu Ser Glu Glu Glu Leu
            660                 665                 670

Ser Trp Ser Glu Asp Glu Val Gln Leu Ile Glu Asp Asp Gly Gln Tyr
        675                 680                 685

Glu Val Asp Glu Thr Ser Val Ser Val Asn Val Glu Gln Asp Ile Gln
    690                 695                 700

Gly Ser Pro Gln Asp Val Val Asp Pro Gln Ala Leu Lys Val Met Leu
705                 710                 715                 720

Gln Glu Leu Ala Glu Lys Asn Tyr Ser Met Arg Asn Lys Leu Phe Val
                725                 730                 735

Phe Pro Glu Val Val Lys Ala Asp Ser Val Ile Asp Leu Tyr Leu Asn
            740                 745                 750

Arg Asp Leu Thr Ala Leu Ala Asn Glu Pro Asp Val Val Ile Lys Gly
        755                 760                 765

Ala Phe Asn Gly Trp Lys Trp Arg Leu Phe Thr Glu Arg Leu His Lys
    770                 775                 780

Ser Asp Leu Gly Gly Val Trp Trp Ser Cys Lys Leu Tyr Ile Pro Lys
785                 790                 795                 800

Glu Ala Tyr Arg Leu Asp Phe Val Phe Phe Asn Gly Arg Thr Val Tyr
                805                 810                 815

Glu Asn Asn Gly Asn Asn Asp Phe Cys Ile Gly Ile Glu Gly Thr Met
            820                 825                 830

Asn Glu Asp Leu Phe Glu Asp Phe Leu Val Lys Glu Lys Gln Arg Glu
        835                 840                 845
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145                 150                 155                 160

Lys Lys Glu Val Asp Ala Ala Asp Lys Ala Arg Val Lys Glu Asp Ala
                165                 170                 175

Phe Glu Leu Asp Leu Pro Ala Thr Thr Leu Arg Ser Val Ile Val Asp
            180                 185                 190

Val Met Asp His Asn Gly Thr Val Gln Glu Thr Leu Arg Ser Val Ile
        195                 200                 205

Val Asp Val Met Asp His Asn Gly Thr Val Gln Glu Thr Leu Arg Ser
    210                 215                 220

Val Ile Val Asp Val Met Asp Asp Ala Ala Asp Lys Ala Arg Val Glu
225                 230                 235                 240

Glu Asp Val Phe Glu Leu Asp Leu Ser Gly Asn Ile Ser Ser Ser Ala
                245                 250                 255

Thr Thr Val Glu Leu Asp Ala Val Asp Glu Val Gly Pro Val Gln Asp
            260                 265                 270

Lys Phe Glu Ala Thr Ser Ser Gly Asn Val Ser Asn Ser Ala Thr Val
        275                 280                 285

Arg Glu Val Asp Ala Ser Asp Glu Ala Gly Asn Asp Gln Gly Ile Phe
    290                 295                 300

Arg Ala Asp Leu Ser Gly Asn Val Phe Ser Ser Ser Thr Thr Val Glu
305                 310                 315                 320

Val Gly Ala Val Asp Glu Ala Gly Ser Ile Lys Asp Arg Phe Glu Thr
                325                 330                 335

Asp Ser Ser Gly Asn Val Ser Thr Ser Ala Pro Met Trp Asp Ala Ile
            340                 345                 350

Asp Glu Thr Val Ala Asp Gln Asp Thr Phe Glu Ala Asp Leu Ser Gly
        355                 360                 365

Asn Ala Ser Ser Cys Ala Thr Tyr Arg Glu Val Asp Asp Val Val Asp
    370                 375                 380

Glu Thr Arg Ser Glu Glu Glu Thr Phe Ala Met Asp Leu Phe Ala Ser
385                 390                 395                 400

Glu Ser Gly His Glu Lys His Met Ala Val Asp Tyr Val Gly Glu Ala
                405                 410                 415

Thr Asp Glu Glu Glu Thr Tyr Gln Gln Gln Tyr Pro Val Pro Ser Ser
            420                 425                 430

Phe Ser Met Trp Asp Lys Ala Ile Ala Lys Thr Gly Val Ser Leu Asn
        435                 440                 445

Pro Glu Leu Arg Leu Val Arg Val Glu Glu Gln Gly Lys Val Asn Phe
    450                 455                 460

Ser Asp Lys Lys Asp Leu Ser Ile Asp Asp Leu Pro Gly Gln Asn Gln
465                 470                 475                 480

Ser Ile Ile Gly Ser Tyr Lys Gln Asp Lys Ser Ile Ala Asp Val Ala
                485                 490                 495
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185 1190 1195 1200

Val Val Thr Ser Leu Ser Arg Ala Ile Gln Asp Leu Gly His Thr Val
1205 1210 1215

Glu Val Ile Leu Pro Lys Tyr Asp Cys Leu Asn Gln Ser Ser Val Lys
1220 1225 1230

Asp Leu His Leu Tyr Gln Ser Phe Ser Trp Gly Gly Thr Glu Ile Lys
1235 1240 1245

Val Trp Val Gly Arg Val Glu Asp Leu Thr Val Tyr Phe Leu Glu Pro
1250 1255 1260

Gln Asn Gly Met Phe Gly Val Gly Cys Val Tyr Gly Arg Asn Asp Asp
265 1270 1275 1280

Arg Arg Phe Gly Phe Phe Cys His Ser Ala Leu Glu Phe Ile Leu Gln
1285 1290 1295

Asn Glu Phe Ser Pro His Ile Ile His Cys His Asp Trp Ser Ser Ala
1300 1305 1310

Pro Val Ala Trp Leu Tyr Lys Glu His Tyr Ser Gln Ser Arg Met Ala
1315 1320 1325

Ser Thr Arg Val Val Phe Thr Ile His Asn Leu Glu Phe Gly Ala His
1330 1335 1340

Tyr Ile Gly Lys Ala Met Thr Tyr Cys Asp Lys Ala Thr Thr Val Ser
345 1350 1355 1360

Pro Thr Tyr Ser Arg Asp Val Ala Gly His Gly Ala Ile Ala Pro His
1365 1370 1375

Arg Glu Lys Phe Tyr Gly Ile Leu Asn Gly Ile Asp Pro Asp Ile Trp
1380 1385 1390

Asp Pro Tyr Thr Asp Asn Phe Ile Pro Val Pro Tyr Thr Cys Glu Asn
1395 1400 1405

Val Val Glu Gly Lys Arg Ala Ala Lys Arg Ala Leu Gln Gln Lys Phe
1410 1415 1420

Gly Leu Gln Gln Thr Asp Val Pro Ile Val Gly Ile Ile Thr Arg Leu
425 1430 1435 1440

Thr Ala Gln Lys Gly Ile His Leu Ile Lys His Ala Ile His Arg Thr
1445 1450 1455

Leu Glu Ser Asn Gly His Val Val Leu Leu Gly Ser Ala Pro Asp His
1460 1465 1470

Arg Ile Gln Gly Asp Phe Cys Arg Leu Ala Asp Ala Leu His Gly Val
1475 1480 1485

Tyr His Gly Arg Val Lys Leu Val Leu Thr Tyr Asp Glu Pro Leu Ser
1490 1495 1500

His Leu Ile Tyr Ala Gly Ser Asp Phe Ile Ile Val Pro Ser Ile Phe
505 1510 1515 1520

Glu Pro Cys Gly Leu Thr Gln Leu Val Ala Met Arg Tyr Gly Ser Ile
1525 1530 1535

Leu Glu Lys Leu Ala Met Glu Glu Ala Glu Arg Arg Thr Gln Thr Glu
850 855 860

Glu Gln Arg Arg Arg Lys Glu Ala Arg Ala Ala Asp Glu Ala Val Arg
865 870 875 880

Ala Gln Ala Lys Ala Glu Ile Glu Ile Lys Lys Lys Lys Leu Gln Ser
885 890 895

Met Leu Ser Leu Ala Arg Thr Cys Val Asp Asn Leu Trp Tyr Ile Glu
900 905 910

Ala Ser Thr Asp Thr Arg Gly Asp Thr Ile Arg Leu Tyr Tyr Asn Arg
915 920 925

Asn Ser Arg Pro Leu Ala His Ser Thr Glu Ile Trp Met His Gly Gly
930 935 940

Tyr Asn Asn Trp Thr Asp Gly Leu Ser Ile Val Glu Ser Phe Val Lys
945 950 955 960

Cys Asn Asp Lys Asp Gly Asp Trp Trp Tyr Ala Asp Val Ile Pro Pro
965 970 975

Glu Lys Ala Leu Val Leu Asp Trp Val Phe Ala Asp Gly Pro Ala Gly
980 985 990

Asn Ala Arg Asn Tyr Asp Asn Asn Ala Arg Gln Asp Phe His Ala Ile
995 1000 1005

Leu Pro Asn Asn Asn Val Thr Glu Glu Gly Phe Trp Ala Gln Glu Glu
1010 1015 1020

Gln Asn Ile Tyr Thr Arg Leu Leu Gln Glu Arg Arg Glu Lys Glu Glu
025 1030 1035 1040

Thr Met Lys Arg Lys Ala Glu Arg Ser Ala Asn Ile Lys Ala Glu Met
1045 1050 1055

Lys Ala Lys Thr Met Arg Arg Phe Leu Leu Ser Gln Lys His Ile Val
1060 1065 1070

Tyr Thr Glu Pro Leu Glu Ile Arg Ala Gly Thr Thr Val Asp Val Leu
1075 1080 1085

Tyr Asn Pro Ser Asn Thr Val Leu Asn Gly Lys Ser Glu Gly Trp Phe
1090 1095 1100

Arg Cys Ser Phe Asn Leu Trp Met His Ser Ser Gly Ala Leu Pro Pro
105 1110 1115 1120

Gln Lys Met Val Lys Ser Gly Asp Gly Pro Leu Leu Lys Ala Thr Val
1125 1130 1135

Asp Val Pro Pro Asp Ala Tyr Met Met Asp Phe Val Phe Ser Glu Trp
1140 1145 1150

Glu Glu Asp Gly Ile Tyr Asp Asn Arg Asn Gly Met Asp Tyr His Ile
1155 1160 1165

Pro Val Ser Asp Ser Ile Glu Thr Glu Asn Tyr Met Arg Ile Ile His
1170 1175 1180

Ile Ala Val Glu Met Ala Pro Val Ala Lys Val Gly Gly Leu Gly Asp

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gca cta aag gtg atg ctg caa gaa ctc gct gag aaa aat tat tcg atg   480
Ala Leu Lys Val Met Leu Gln Glu Leu Ala Glu Lys Asn Tyr Ser Met
145                 150                 155                 160

agg aac aag ctg ttt gtt ttt cca gag gta gtg aaa gct gat tca gtt   528
Arg Asn Lys Leu Phe Val Phe Pro Glu Val Val Lys Ala Asp Ser Val
                165                 170                 175

att gat ctt tat tta aat cgt gac cta aca gct ttg gcg aat gaa ccc   576
Ile Asp Leu Tyr Leu Asn Arg Asp Leu Thr Ala Leu Ala Asn Glu Pro
            180                 185                 190

gat gtc gtc atc aaa gga gca ttc aat ggt tgg aaa tgg agg ctt ttc   624
Asp Val Val Ile Lys Gly Ala Phe Asn Gly Trp Lys Trp Arg Leu Phe
        195                 200                 205

act gaa aga ttg cac aag agt gac ctt gga ggg gtt tgg tgg tct tgc   672
Thr Glu Arg Leu His Lys Ser Asp Leu Gly Gly Val Trp Trp Ser Cys
    210                 215                 220

aaa ctg tac ata ccc aag gag gcc tac aga tta gac ttt gtg ttc ttc   720
Lys Leu Tyr Ile Pro Lys Glu Ala Tyr Arg Leu Asp Phe Val Phe Phe
225                 230                 235                 240

aac ggt cgc acg gtc tat gag aac aat ggc aac aat gat ttc tgt ata   768
Asn Gly Arg Thr Val Tyr Glu Asn Asn Gly Asn Asn Asp Phe Cys Ile
                245                 250                 255

gga ata gaa ggc act atg aat gaa gat ctg ttt gag gat ttc ttg gtt   816
Gly Ile Glu Gly Thr Met Asn Glu Asp Leu Phe Glu Asp Phe Leu Val
            260                 265                 270

aaa gaa aag caa agg gag ctt gag aaa ctt gcc atg gaa gaa gct gaa   864
Lys Glu Lys Gln Arg Glu Leu Glu Lys Leu Ala Met Glu Glu Ala Glu
        275                 280                 285

agg agg aca cag act gaa gaa cag cgg cga aga aag gaa gca agg gct   912
Arg Arg Thr Gln Thr Glu Glu Gln Arg Arg Arg Lys Glu Ala Arg Ala
    290                 295                 300

gca gat gaa gct gtc agg gca caa gcg aag gcc gag ata gag atc aag   960
Ala Asp Glu Ala Val Arg Ala Gln Ala Lys Ala Glu Ile Glu Ile Lys
305                 310                 315                 320

aag aaa aaa ttg caa agt atg ttg agt ttg gcc aga aca tgt gtt gat  1008
Lys Lys Lys Leu Gln Ser Met Leu Ser Leu Ala Arg Thr Cys Val Asp
                325                 330                 335

aat ttg tgg tac ata gag gct agc aca gat aca aga gga gat act atc  1056
Asn Leu Trp Tyr Ile Glu Ala Ser Thr Asp Thr Arg Gly Asp Thr Ile
            340                 345                 350

agg tta tat tat aac aga aac tcg agg cca ctt gcg cat agt act gag  1104
Arg Leu Tyr Tyr Asn Arg Asn Ser Arg Pro Leu Ala His Ser Thr Glu
        355                 360                 365

att tgg atg cat ggt ggt tac aac aat tgg tca gat gga ctc tct att  1152
Ile Trp Met His Gly Gly Tyr Asn Asn Trp Ser Asp Gly Leu Ser Ile
    370                 375                 380

gtt gaa agc ttt gtc aag tgc aat gac aaa gac ggc gat tgg tgg tat  1200
Val Glu Ser Phe Val Lys Cys Asn Asp Lys Asp Gly Asp Trp Trp Tyr
385                 390                 395                 400

gca gat gtt att cca cct gaa aag gca ctt gtg ttg gac tgg gtt ttt  1248
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Pro Ile Val Arg Lys Thr Gly Gly Leu His Asp Thr Val Phe Asp Val
            1540                1545                1550

Asp Asn Asp Lys Asp Arg Ala Arg Ser Leu Gly Leu Glu Pro Asn Gly
        1555                1560                1565

Phe Ser Phe Asp Gly Ala Asp Ser Asn Gly Val Asp Tyr Ala Leu Asn
    1570                1575                1580

Arg Ala Ile Gly Ala Trp Phe Asp Ala Arg Asp Trp Phe His Ser Leu
585                 1590                1595                1600

Cys Lys Arg Val Met Glu Gln Asp Trp Ser Trp Asn Arg Pro Ala Leu
                1605                1610                1615

Asp Tyr Ile Glu Leu Tyr His Ala Ala Arg Lys Phe
            1620                1625


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  1               5                  10                  15

tcc gag aaa act gat gag gat gcg ctt cat gta aag ttt aat gtt gac   96
Ser Glu Lys Thr Asp Glu Asp Ala Leu His Val Lys Phe Asn Val Asp
             20                  25                  30

aat gtg ttg cgg aag cat cag gca gat aga acc caa gca gtg gaa aag   144
Asn Val Leu Arg Lys His Gln Ala Asp Arg Thr Gln Ala Val Glu Lys
         35                  40                  45

aaa act tgg aag aaa gtt gat gag gaa cat ctt tac atg act gaa cat   192
Lys Thr Trp Lys Lys Val Asp Glu Glu His Leu Tyr Met Thr Glu His
     50                  55                  60

cag aaa cgt gct gcc gaa gga cag atg gta gtt aac gag gat gag ctt   240
Gln Lys Arg Ala Ala Glu Gly Gln Met Val Val Asn Glu Asp Glu Leu
 65                  70                  75                  80

tct ata act gaa att gga atg ggg aga ggt gat aaa att cag cat gtg   288
Ser Ile Thr Glu Ile Gly Met Gly Arg Gly Asp Lys Ile Gln His Val
                 85                  90                  95

ctt tct gag gaa gag ctt tca tgg tct gaa gat gaa gtg cag tta att   336
Leu Ser Glu Glu Glu Leu Ser Trp Ser Glu Asp Glu Val Gln Leu Ile
            100                 105                 110

gag gat gat gga caa tat gaa gtt gac gag acc tct gtg tcc gtt aac   384
Glu Asp Asp Gly Gln Tyr Glu Val Asp Glu Thr Ser Val Ser Val Asn
        115                 120                 125

gtt gaa caa gat atc cag ggg tca cca cag gat gtt gtg gat ccg caa   432
Val Glu Gln Asp Ile Gln Gly Ser Pro Gln Asp Val Val Asp Pro Gln
    130                 135                 140
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            660                 665                 670

tgg ggt ggt aca gaa ata aaa gta tgg gtt gga cga gtc gaa gac ctg   2064
Trp Gly Gly Thr Glu Ile Lys Val Trp Val Gly Arg Val Glu Asp Leu
        675                 680                 685

acc gtt tac ttc ctg gaa cct caa aat ggg atg ttt ggc gtt gga tgt   2112
Thr Val Tyr Phe Leu Glu Pro Gln Asn Gly Met Phe Gly Val Gly Cys
    690                 695                 700

gta tat gga agg aat gat gac cgc aga ttt ggg ttc ttc tgt cat tct   2160
Val Tyr Gly Arg Asn Asp Asp Arg Arg Phe Gly Phe Phe Cys His Ser
705                 710                 715                 720

gct cta gag ttt atc ctc cag aat gaa ttt tct cca cat ata ata cat   2208
Ala Leu Glu Phe Ile Leu Gln Asn Glu Phe Ser Pro His Ile Ile His
                725                 730                 735

tgc cat gat tgg tca agt gct ccg gtc gcc tgg cta tat aag gaa cac   2256
Cys His Asp Trp Ser Ser Ala Pro Val Ala Trp Leu Tyr Lys Glu His
            740                 745                 750

tat tcc caa tcc aga atg gca agc act cgg gtt gta ttt acc atc cac   2304
Tyr Ser Gln Ser Arg Met Ala Ser Thr Arg Val Val Phe Thr Ile His
        755                 760                 765

aat ctt gaa ttt gga gca cat tat att ggt aaa gca atg aca tac tgt   2352
Asn Leu Glu Phe Gly Ala His Tyr Ile Gly Lys Ala Met Thr Tyr Cys
    770                 775                 780

gat aaa gcc aca act gtt tct cct aca tat tca agg gac gtg gca ggc   2400
Asp Lys Ala Thr Thr Val Ser Pro Thr Tyr Ser Arg Asp Val Ala Gly
785                 790                 795                 800

cat ggc gcc att gct cct cat cgt gag aaa ttc tac ggc att ctc aat   2448
His Gly Ala Ile Ala Pro His Arg Glu Lys Phe Tyr Gly Ile Leu Asn
                805                 810                 815

gga att gat cca gat atc tgg gat ccg tac act gac aat ttt atc ccg   2496
Gly Ile Asp Pro Asp Ile Trp Asp Pro Tyr Thr Asp Asn Phe Ile Pro
            820                 825                 830

gtc cct tat act tgt gag aat gtt gtc gaa ggc aag agg gct gca aaa   2544
Val Pro Tyr Thr Cys Glu Asn Val Val Glu Gly Lys Arg Ala Ala Lys
        835                 840                 845

agg gcc ttg cag cag aag ttt gga tta cag caa act gat gtc cct att   2592
Arg Ala Leu Gln Gln Lys Phe Gly Leu Gln Gln Thr Asp Val Pro Ile
    850                 855                 860

gtc gga atc atc acc cgt ctg aca gca cag aag gga atc cac ctc atc   2640
Val Gly Ile Ile Thr Arg Leu Thr Ala Gln Lys Gly Ile His Leu Ile
865                 870                 875                 880

aag cac gca att cac cga acc ctc gag agc aat gga caa gtg gtt ttg   2688
Lys His Ala Ile His Arg Thr Leu Glu Ser Asn Gly Gln Val Val Leu
                885                 890                 895

ctt ggt tca gct cca gat cat cga ata caa ggc gat ttt tgc aga ttg   2736
Leu Gly Ser Ala Pro Asp His Arg Ile Gln Gly Asp Phe Cys Arg Leu
            900                 905                 910

gcc gat gct ctt cac ggt gtt tac cat ggt agg gtg aag ctt gtt cta   2784
Ala Asp Ala Leu His Gly Val Tyr His Gly Arg Val Lys Leu Val Leu
        915                 920                 925
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Ala Asp Val Ile Pro Pro Glu Lys Ala Leu Val Leu Asp Trp Val Phe
                405                 410                 415

gct gat ggg cca gct ggg aat gca agg aac tat gac aac aat gct cga  1296
Ala Asp Gly Pro Ala Gly Asn Ala Arg Asn Tyr Asp Asn Asn Ala Arg
            420                 425                 430

caa gat ttc cat gct att ctt ccg aac aac aat gta acc gag gaa ggc  1344
Gln Asp Phe His Ala Ile Leu Pro Asn Asn Asn Val Thr Glu Glu Gly
        435                 440                 445

ttc tgg gcg caa gag gag caa aac atc tat aca agg ctt ctg caa gaa  1392
Phe Trp Ala Gln Glu Glu Gln Asn Ile Tyr Thr Arg Leu Leu Gln Glu
    450                 455                 460

agg aga gaa aag gaa gaa acc atg aaa aga aag gct gag aga agt gca  1440
Arg Arg Glu Lys Glu Glu Thr Met Lys Arg Lys Ala Glu Arg Ser Ala
465                 470                 475                 480

aat atc aaa gct gag atg aag gca aaa act atg cga agg ttt ctg ctt  1488
Asn Ile Lys Ala Glu Met Lys Ala Lys Thr Met Arg Arg Phe Leu Leu
                485                 490                 495

tcc cag aaa cac att gtt tat acc cga acc gnc ttg aaa tac gtg ccc  1536
Ser Gln Lys His Ile Val Tyr Thr Arg Thr Xaa Leu Lys Tyr Val Pro
            500                 505                 510

gga acc aca gtg gat gtg cta tac aat ccc tct aac aca gtg cta aat  1584
Gly Thr Thr Val Asp Val Leu Tyr Asn Pro Ser Asn Thr Val Leu Asn
        515                 520                 525

gga aag tcg gag ggt tgg ttt aga tgc tcc ttt aac ctt tgg atg cat  1632
Gly Lys Ser Glu Gly Trp Phe Arg Cys Ser Phe Asn Leu Trp Met His
    530                 535                 540

tca agt ggg gca ttg cca ccc cag aag atg gtg aaa tca ggg gat ggg  1680
Ser Ser Gly Ala Leu Pro Pro Gln Lys Met Val Lys Ser Gly Asp Gly
545                 550                 555                 560

ccg ctc tta aaa gca aca gtt gat gtt cca ccg gat gcc tat atg atg  1728
Pro Leu Leu Lys Ala Thr Val Asp Val Pro Pro Asp Ala Tyr Met Met
                565                 570                 575

gac ttt gtt ttc tcc gag tgg gaa gaa gat ggg atc tat gac aac agg  1776
Asp Phe Val Phe Ser Glu Trp Glu Glu Asp Gly Ile Tyr Asp Asn Arg
            580                 585                 590

aat ggg atg gac tat cat att cct gtt tct gat tca att gaa aca gag  1824
Asn Gly Met Asp Tyr His Ile Pro Val Ser Asp Ser Ile Glu Thr Glu
        595                 600                 605

aat tac atg cgt att atc cac att gcc gtt gag atg gcc ccc gtt gca  1872
Asn Tyr Met Arg Ile Ile His Ile Ala Val Glu Met Ala Pro Val Ala
    610                 615                 620

aag gtt gga ggt ctt ggg gat gtt gtt aca agt ctt tca cgt gcc att  1920
Lys Val Gly Gly Leu Gly Asp Val Val Thr Ser Leu Ser Arg Ala Ile
625                 630                 635                 640

caa gat cta gga cat act gtc gag gtt att ctc ccg aag tac gac tgt  1968
Gln Asp Leu Gly His Thr Val Glu Val Ile Leu Pro Lys Tyr Asp Cys
                645                 650                 655

ttg aac caa agc agt gtc aag gat tta cat tta tat caa agt ttt tct  2016
Leu Asn Gln Ser Ser Val Lys Asp Leu His Leu Tyr Gln Ser Phe Ser
```

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Lys Thr Trp Lys Lys Val Asp Glu Glu His Leu Tyr Met Thr Glu His
 50                  55                  60

Gln Lys Arg Ala Ala Glu Gly Gln Met Val Val Asn Glu Asp Glu Leu
65                  70                  75                  80

Ser Ile Thr Glu Ile Gly Met Gly Arg Gly Asp Lys Ile Gln His Val
                85                  90                  95

Leu Ser Glu Glu Glu Leu Ser Trp Ser Glu Asp Glu Val Gln Leu Ile
            100                 105                 110

Glu Asp Asp Gly Gln Tyr Glu Val Asp Glu Thr Ser Val Ser Val Asn
        115                 120                 125

Val Glu Gln Asp Ile Gln Gly Ser Pro Gln Asp Val Val Asp Pro Gln
    130                 135                 140

Ala Leu Lys Val Met Leu Gln Glu Leu Ala Glu Lys Asn Tyr Ser Met
145                 150                 155                 160

Arg Asn Lys Leu Phe Val Phe Pro Glu Val Val Lys Ala Asp Ser Val
                165                 170                 175

Ile Asp Leu Tyr Leu Asn Arg Asp Leu Thr Ala Leu Ala Asn Glu Pro
            180                 185                 190

Asp Val Val Ile Lys Gly Ala Phe Asn Gly Trp Lys Trp Arg Leu Phe
        195                 200                 205

Thr Glu Arg Leu His Lys Ser Asp Leu Gly Gly Val Trp Trp Ser Cys
    210                 215                 220

Lys Leu Tyr Ile Pro Lys Glu Ala Tyr Arg Leu Asp Phe Val Phe Phe
225                 230                 235                 240

Asn Gly Arg Thr Val Tyr Glu Asn Asn Gly Asn Asn Asp Phe Cys Ile
                245                 250                 255

Gly Ile Glu Gly Thr Met Asn Glu Asp Leu Phe Glu Asp Phe Leu Val
            260                 265                 270

Lys Glu Lys Gln Arg Glu Leu Glu Lys Leu Ala Met Glu Glu Ala Glu
        275                 280                 285

Arg Arg Thr Gln Thr Glu Glu Gln Arg Arg Arg Lys Glu Ala Arg Ala
    290                 295                 300

Ala Asp Glu Ala Val Arg Ala Gln Ala Lys Ala Glu Ile Glu Ile Lys
305                 310                 315                 320

Lys Lys Lys Leu Gln Ser Met Leu Ser Leu Ala Arg Thr Cys Val Asp
                325                 330                 335

Asn Leu Trp Tyr Ile Glu Ala Ser Thr Asp Thr Arg Gly Asp Thr Ile
            340                 345                 350

Arg Leu Tyr Tyr Asn Arg Asn Ser Arg Pro Leu Ala His Ser Thr Glu
        355                 360                 365

Ile Trp Met His Gly Gly Tyr Asn Asn Trp Ser Asp Gly Leu Ser Ile
    370                 375                 380

Val Glu Ser Phe Val Lys Cys Asn Asp Lys Asp Gly Asp Trp Trp Tyr
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acc tac gat gag cct ctt tct cac ctg ata tac gct ggc tcc gac ttc   2832
Thr Tyr Asp Glu Pro Leu Ser His Leu Ile Tyr Ala Gly Ser Asp Phe
    930                 935                 940

att att gtc cct tca atc ttt gaa ccc tgt ggc tta aca caa ctt gtt   2880
Ile Ile Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln Leu Val
945                 950                 955                 960

gcc atg cgt tat gga tcg atc cct ata gtt cgg aaa acc gga gga ctt   2928
Ala Met Arg Tyr Gly Ser Ile Pro Ile Val Arg Lys Thr Gly Gly Leu
                965                 970                 975

tac gac act gtc ttc gac gta gac aat gat aag gac cgg gct cgg tct   2976
Tyr Asp Thr Val Phe Asp Val Asp Asn Asp Lys Asp Arg Ala Arg Ser
            980                 985                 990

ctt ggt ctt gaa cca aat ggg ttc agt ttc gac gga gcc gac agc aat   3024
Leu Gly Leu Glu Pro Asn Gly Phe Ser Phe Asp Gly Ala Asp Ser Asn
        995                 1000                1005

ggc gtg gat tat gcc ctc aac aga gca atc ggc gct tgg ttc gat gcc   3072
Gly Val Asp Tyr Ala Leu Asn Arg Ala Ile Gly Ala Trp Phe Asp Ala
   1010                1015                1020

cgt gat tgg ttc cac tcc ctg tgt aag agg gtc atg gag caa gac tgg   3120
Arg Asp Trp Phe His Ser Leu Cys Lys Arg Val Met Glu Gln Asp Trp
1025                1030                1035                1040

tcg tgg aac cgg cct gca ctg gac tac att gaa ttg tac cat gcc gct   3168
Ser Trp Asn Arg Pro Ala Leu Asp Tyr Ile Glu Leu Tyr His Ala Ala
               1045                1050                1055

cga aaa ttc tgacacccaa ctgaaccaat ggcaagaaca agcgcattgt           3217
Arg Lys Phe

gggatcgact acagtcatac agggctgtgc agatcgtctt gcttcagtta gtgccctctt 3277

cagttagttc caagcgcact acagtcgtac atagctgagg atcctcttgc ctcctccacc 3337

aggggaaaca aagcagaaat gcataagtgc attgggaaga cttttatgta tattgttaaa 3397

ttttttcttt tcttttcctt ccctgcacct ggaaatggtt aagcgcatcg ccgagataag 3457

aaccacagta acattctgtg agtagctttg tatattctct catcttgtga aaactaatgt 3517

gcatgttagg ctctctgatc atgtggaagc tttgttatat gttacttatg gttatatggt 3577

atacatcaat gatatttaca tttgtggaaa aaaaaaaaaa aaaa                   3621
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<210> 10
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<212> PRT
<213> Triticum aestivum

<400> 10

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Asp Ala Leu Tyr Val Asn Gly Leu Glu Ala Lys Glu Gly Asp His Thr
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Ser Glu Lys Thr Asp Glu Asp Ala Leu His Val Lys Phe Asn Val Asp
             20                  25                  30

Asn Val Leu Arg Lys His Gln Ala Asp Arg Thr Gln Ala Val Glu Lys
         35                  40                  45
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Cys His Asp Trp Ser Ser Ala Pro Val Ala Trp Leu Tyr Lys Glu His
            740                 745                 750

Tyr Ser Gln Ser Arg Met Ala Ser Thr Arg Val Val Phe Thr Ile His
        755                 760                 765

Asn Leu Glu Phe Gly Ala His Tyr Ile Gly Lys Ala Met Thr Tyr Cys
    770                 775                 780

Asp Lys Ala Thr Thr Val Ser Pro Thr Tyr Ser Arg Asp Val Ala Gly
785                 790                 795                 800

His Gly Ala Ile Ala Pro His Arg Glu Lys Phe Tyr Gly Ile Leu Asn
                805                 810                 815

Gly Ile Asp Pro Asp Ile Trp Asp Pro Tyr Thr Asp Asn Phe Ile Pro
            820                 825                 830

Val Pro Tyr Thr Cys Glu Asn Val Val Glu Gly Lys Arg Ala Ala Lys
        835                 840                 845

Arg Ala Leu Gln Gln Lys Phe Gly Leu Gln Gln Thr Asp Val Pro Ile
    850                 855                 860

Val Gly Ile Ile Thr Arg Leu Thr Ala Gln Lys Gly Ile His Leu Ile
865                 870                 875                 880

Lys His Ala Ile His Arg Thr Leu Glu Ser Asn Gly Gln Val Val Leu
                885                 890                 895

Leu Gly Ser Ala Pro Asp His Arg Ile Gln Gly Asp Phe Cys Arg Leu
            900                 905                 910

Ala Asp Ala Leu His Gly Val Tyr His Gly Arg Val Lys Leu Val Leu
        915                 920                 925

Thr Tyr Asp Glu Pro Leu Ser His Leu Ile Tyr Ala Gly Ser Asp Phe
    930                 935                 940

Ile Ile Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln Leu Val
945                 950                 955                 960

Ala Met Arg Tyr Gly Ser Ile Pro Ile Val Arg Lys Thr Gly Gly Leu
                965                 970                 975

Tyr Asp Thr Val Phe Asp Val Asp Asn Asp Lys Asp Arg Ala Arg Ser
            980                 985                 990

Leu Gly Leu Glu Pro Asn Gly Phe Ser Phe Asp Gly Ala Asp Ser Asn
        995                1000                1005

Gly Val Asp Tyr Ala Leu Asn Arg Ala Ile Gly Ala Trp Phe Asp Ala
   1010                1015                1020

Arg Asp Trp Phe His Ser Leu Cys Lys Arg Val Met Glu Gln Asp Trp
1025                1030                1035                1040

Ser Trp Asn Arg Pro Ala Leu Asp Tyr Ile Glu Leu Tyr His Ala Ala
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Arg Lys Phe
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420 425 430

Gln Asp Phe His Ala Ile Leu Pro Asn Asn Asn Val Thr Glu Glu Gly
435 440 445

Phe Trp Ala Gln Glu Glu Gln Asn Ile Tyr Thr Arg Leu Leu Gln Glu
450 455 460

Arg Arg Glu Lys Glu Glu Thr Met Lys Arg Lys Ala Glu Arg Ser Ala
465 470 475 480

Asn Ile Lys Ala Glu Met Lys Ala Lys Thr Met Arg Arg Phe Leu Leu
485 490 495

Ser Gln Lys His Ile Val Tyr Thr Arg Thr Xaa Leu Lys Tyr Val Pro
500 505 510

Gly Thr Thr Val Asp Val Leu Tyr Asn Pro Ser Asn Thr Val Leu Asn
515 520 525

Gly Lys Ser Glu Gly Trp Phe Arg Cys Ser Phe Asn Leu Trp Met His
530 535 540

Ser Ser Gly Ala Leu Pro Pro Gln Lys Met Val Lys Ser Gly Asp Gly
545 550 555 560

Pro Leu Leu Lys Ala Thr Val Asp Val Pro Pro Asp Ala Tyr Met Met
565 570 575

Asp Phe Val Phe Ser Glu Trp Glu Glu Asp Gly Ile Tyr Asp Asn Arg
580 585 590

Asn Gly Met Asp Tyr His Ile Pro Val Ser Asp Ser Ile Glu Thr Glu
595 600 605

Asn Tyr Met Arg Ile Ile His Ile Ala Val Glu Met Ala Pro Val Ala
610 615 620

Lys Val Gly Gly Leu Gly Asp Val Val Thr Ser Leu Ser Arg Ala Ile
625 630 635 640

Gln Asp Leu Gly His Thr Val Glu Val Ile Leu Pro Lys Tyr Asp Cys
645 650 655

Leu Asn Gln Ser Ser Val Lys Asp Leu His Leu Tyr Gln Ser Phe Ser
660 665 670

Trp Gly Gly Thr Glu Ile Lys Val Trp Val Gly Arg Val Glu Asp Leu
675 680 685

Thr Val Tyr Phe Leu Glu Pro Gln Asn Gly Met Phe Gly Val Gly Cys
690 695 700

Val Tyr Gly Arg Asn Asp Asp Arg Arg Phe Gly Phe Phe Cys His Ser
705 710 715 720

Ala Leu Glu Phe Ile Leu Gln Asn Glu Phe Ser Pro His Ile Ile His
725 730 735

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<211> 1032
<212> DNA
<213> Triticum sp.

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<212> DNA
<213> Triticum sp.

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gagatctcca cgccagagcg ttgtattcca attttagttc tttccccgtg aggaggggag 180

gctaggcggg cgaggcagag gggatagggc agtcgccgct gcgtggtgga ctgactggtg 240

tggtgggtgg tgggttttgc gggcggggtt tagtaggttc ccggaaatgg agatggctct 300

ccggccacgg agccctctgt gccctcggag cagtcagccg ctcgtcgtcg tccggccggc 360

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tgagctggaa ttcatactgc ttaaaacgac gtgattttaa ttgctggaag aggtaaagaa 660

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<213> Triticum sp.

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<211> 871
<212> DNA
<213> Triticum sp.

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tcctacctgg cttcggaact gaaagaactg g 871

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<211> 1592
<212> DNA
<213> Triticum sp.

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caaggccaca gatagtttta tgcttaacta tgtgtttcat actacttcag gtcccttata 480

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<212> DNA
<213> Triticum sp.

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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 19
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<210> 20
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 20
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1 5 10

<210> 21
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 21
Ala Thr Gly Lys Lys Asp Ala Leu
1 5

<210> 22
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 22
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1 5

<210> 23
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 23
Ala Ala Gly Lys Lys Asp Ala Arg Val Asp Asp Asp Ala Ala
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gaatccacct catcaagcac gcaattcacc gaaccctcga aagcaacgga caggttcatc 660

atcccttgtg aacgaataaa catcaaacgt tttgtttata aaaagttgct tactatttgt 720

ttttgtttac ttcaaaacaa aagtctgaaa atgaagtgtt tggttcctag gtggttttgc 780

ttggttcagc tccagatcat cgaatacaag gcgatttttg cagattggcc gatgctcttc 840

acggtgttta ccacggtagg gtgaagcttg ttctaaccta cgatgagcct ctttctcacc 900

tggtgagctc caatatccta cacaccatct agccagccct tcattatggg agctggagac 960

tactttataa tttaggttga tgatcgatca tgctgcagat atacgctggc tccgacttca 1020

ttattgtccc ttcaatcttc gaaccctgtg gcttaacaca acttgttgcc atgcgttatg 1080

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actgatttgc atatgtcgag ctgttggaca tgaaatggaa actatccttt ggtatcgcag 1200

gactttacga cactgtcttc gacgtagaca atgataagga ccgggctcgg tctcttggtc 1260

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tccactccct gtgtaagagg gtcatggaac aagactggtc atggaaccgg cccgcactgg 1500

actacattga attgtaccat gccgctcgaa aattctgaca cccaactgaa ccaatggcaa 1560

gaacaagcgc attgtgggat cgagaattcc cg 1592

<210> 17
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 17
Asp Val Gln Leu Val Met Leu Gly Thr Gly
1 5 10

<210> 18
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 18
Ala Ala Gly Lys Lys Asp Ala Gly Ile Asp
1 5 10

<210> 19

<220>
<223> Description of Artificial Sequence:PRIMER

<400> 29
ggaggtcttg gtgatgttgt 20

<210> 30
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PRIMER

<400> 30
cttgaccaat catggcaatg 20

<210> 31
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PRIMER

<400> 31
cattgccatg attggtcaag 20

<210> 32
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PRIMER

<400> 32
accacctgtc cgttccgttg c 21

<210> 33
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PRIMER

<400> 33
gcacggtcta tgagaacaat ggc 23

<210> 34
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PRIMER

<400> 34

<210> 24
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 24
Ala Leu Gly Lys Lys Asp Ala Gly Ile Val Asp Gly Ala
1 5 10

<210> 25
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PRIMER

<400> 25 23
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<210> 26
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PRIMER

<400> 26 23
agtcgttctg ccgtatgatg tcg

<210> 27
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PRIMER

<400> 27 21
ccaagtacca gtggtgaacg c

<210> 28
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PRIMER

<400> 28 19
cggtgggatc caacggccc

<210> 29
<211> 20
<212> DNA
<213> Artificial Sequence

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<210> 35
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 35
Lys Val Gly Gly Leu Gly Asp Val Val Thr Ser Leu Ser Arg Ala Val
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Gln Asp Leu Gly His Asn Val Glu Val
20 25

<210> 36
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 36
Lys Val Gly Gly Leu Gly Asp Val Val Thr Ser Leu Ser Arg Ala Ile
1 5 10 15

Gln Asp Leu Gly His Thr Val Glu Val
20 25

<210> 37
<211> 9024
<212> DNA
<213> Triticum sp.

<400> 37
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gttcccttat tatctcccgt gcaccccggt agcgtaggac aaaaagaaaa aatagaacgg 180

acccaacgtc acaagctcac acatgcccag cgagagaaaa gaaaaatggt gcgacaaaaa 240

aaaggaaacg ggctgagagc cgaaacacat gggctgcgct ttgttcgcta cgaagctctc 300

ccctcgacaa aatatgaatc gcgacgtgat tggatcctat ggtggaaaaa gtgaatgaga 360

ccaaaagaat tctcagctga atgagtttta gcaagactga tcattatatc caacataaat 420

agatttttt tttgcaaaaa taatccaaat ctattagcaa agttcagtag aagtacaaag 480

catctcgaac attataaaca ttacactgag attccaggac caccaaacaa cccactactg 540

ccgcgaaaag aaaaggattc ggaagacaga aattatccaa accacgttcg tccttggttg 600

ttggtctcat tgcgcgctaa acaacctgga cagcagaaga agcaaagcag tgtgcttccg 660

ctccgcagca agaagacaag tcgtcacatg tcagacgccg tcactcaagc aagcaaactg 720

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<210> 38
<211> 11611
<212> DNA
<213> Triticum aestivum

<400> 38
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acaattttgg gatactaaac ctggatgctc agtctactcc catgtgaagt ttcatgaaaa 180

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00385

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C12N 15/54, 15/11; C12N 9/10; C12Q 1/48, 1/68; A01H 1/00, 5/00; C08B 3/02.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
WORLD PATENT INDEX (WPI).

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
GENBANK, EMBL, SWISS-PROTEINS, PIR

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
KW: WPI Starch synthase. Seq id nos 2, 4, 6, 8, 10 and 39-54.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	Li Z *et al* "The localization and expression of the class II starch synthases of wheat" Plant Physiol 1999 Aug 120(4) pp 1147-1156. See the whole document.	1-59.
P, X	GenPept accession no. CAB86618, and GenBank accession no. AJ269502, published 7 April 2000. Gao M and Chibbar R N "Isolation, characterization and expression analysis of starch synthase IIa c DNA from wheat (Triticum aestivum L.)" See the whole document.	1-8, 10-19 and 21 (seq id nos 1-6, 50 and 53)
X; Y	WO 97/45545 A (HOECHST SCHERING AGREVO GmbH) 4 December 1997. See the whole document especially the examples and seq id no 5.	1-8, 10-19, 21-38 and 41-59 (seq id nos 1-6, 50 and 53)

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 June 2000	20 JUN 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer J.H. CHAN Telephone No : (02) 6283 2340

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00385

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9745545	AU	30302/97	BR	9709487	CN	1219970
		CZ	9803890	DE	19621588	EP	907741
		SK	1636/98	ZA	9704657		

END OF ANNEX